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Contents

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VOL. 27, SEC. B. MAY, 1949 NUMBER 5

A NOTE ON THE EFFECT OF PHOSGENE UPON THE INFLAMMABILITY LIMITS OF HEXANE[1]

BY K. J. MCCALLUM AND HELEN M. TRAINOR

Abstract

The inflammability limits of mixtures of phosgene and hexane in air at approximately atmospheric pressure have been investigated. The addition of phosgene causes the upper and lower inflammability limits of hexane to approach each other until they meet at a composition containing 3.4 volume % hexane and 19.1 volume % phosgene.

Introduction

The upper and lower inflammability limits of hexane in air have been reported to be 6.9 and 1.4 volume % (4) and 6.9 and 1.2 volume % (1). This paper reports the effects of the addition of phosgene upon these limits.

Materials and Procedure

The phosgene was of commercial grade and was used as received with no further purification. The material was analyzed by the method of Nenitzescu and Pana (5) to determine the percentage of free chlorine. This was found to be 0.46%. No attempt was made to determine carbon monoxide or other gases.

The hexane used was Eastman Kodak Company's hexane from petroleum. The value of the refractive index at 20° C., using the sodium D line, was 1.37576 as compared to the value of 1.37506 given by Egloff (2).

The inflammability limits of mixtures of phosgene, hexane, and air were determined at room temperature and approximately 1 atm. pressure. The inflammability tests were made in a Pyrex tube, 47.5 mm. inside diameter and 103 cm. long. The method of preparing and mixing the gases of different compositions was the same as that previously described (4). A small gas flame was used as the source of ignition. All observations were made with upward propagation of the flame and with the lower end of the tube open, so that the pressure during the combustion remained close to the atmospheric pressure. Gases of various compositions were tested until compositions were found that were approximately 0.3% on either side of a limit mixture.

[1] *Manuscript received January 10, 1949.*

Contribution from the Department of Chemistry, University of Saskatchewan, Saskatoon, Saskatchewan.

Results and Discussion

In Table I are given the compositions of limit mixtures of phosgene, hexane, and air. The table contains the partial pressures of the gases in the mixtures between which the limits were found to lie. The compositions of the limit mixtures in volume per cent were calculated on the assumption that partial pressure per cent is equal to volume per cent.

TABLE I

INFLAMMABILITY LIMITS FOR PHOSGENE–HEXANE–AIR

Partial pressure of limit mixture, mm. mercury		Barometer height, mm. mercury	Vol. % hexane (mean)	Vol. % phosgene (mean)	Vol. % air (mean)
Hexane	Phosgene				
10.2–10.7	0	705.5	1.5	0	98.5
52 –54	0	709.0	7.5	0	92.5
14.2	46 – 48	710.8	2.0	6.55	91.45
43.0–44.2	43.0– 44.2	715.0	6.1	6.1	87.8
29.6–29.5	115.9–111.9	719.2	4.1	15.8	80.1
15.4–18.4	86.4– 86.0	718.5	2.35	12.0	85.65
24.0–22.8	136.8–137.0	718.5	3.26	19.0	77.74
34.3–37.5	79.3– 79.5	718.5	5.10	11.0	83.9
21.8–24.0	133 –135	718.5	3.20	18.6	78.2
24.0–24.5	136.8–138.2	718.5	3.37	19.1	77.53
26.6	129	718.5	3.70	18.0	78.3

The upper and lower inflammability limits of hexane in air were found to be 7.5 volume % and 1.5 volume %, respectively. The corresponding values reported by McCallum and Graham (4) are 6.9% and 1.4%, respectively, while Coward and Jones (1) report 6.9% and 1.2% for *n*-hexane. The agreement is within the precision of these experiments.

In Fig. 1, the inflammability limits for this system are plotted as volume per cent phosgene against volume per cent hexane. The addition of phosgene to hexane is seen to bring the upper and lower inflammability limits together until eventually they meet, the maximum in the curve occurring at a composition containing 3.4 volume % hexane and 19.1 volume % phosgene.

By combining the data for this system with those for the systems hydrogen cyanide–phosgene–air and hydrogen cyanide–hexane–air (4), the inflammability limits in the quaternary system hydrogen cyanide–hexane–phosgene–air can be calculated assuming that the law of Le Chatelier (3) holds. This law, which has been found to hold in the system hydrogen cyanide–hexane–air, states that mixing two upper or two lower binary limit mixtures will produce an upper or lower limit mixture, respectively, of the resulting ternary system.

The essential assumption is, that just as the law gives the inflammability limits of mixtures of the two inflammable gases, hydrogen cyanide and hexane,

in air, it also gives the limits of mixtures of these gases in a given phosgene–air mixture. The upper and lower inflammability limits of hexane and of hydrogen cyanide, separately, in a mixture containing phosgene and air in a

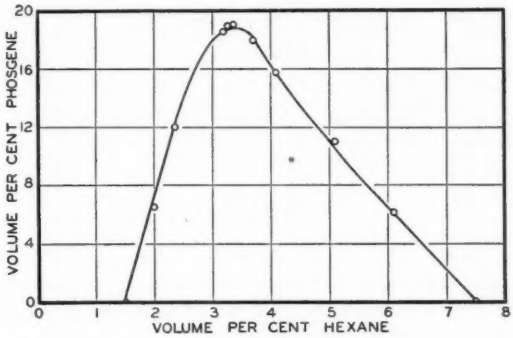

FIG. 1. *Inflammability limits in the system phosgene–hexane–air.*

given ratio, can be obtained from the curves for the systems hydrogen cyanide–phosgene–air, and hexane–phosgene–air. Those compositions of hexane and hydrogen cyanide together in this phosgene–air mixture which are inflammable can be found by joining the points representing these upper and lower limits, respectively, on a plot of volume per cent hexane versus volume per cent hydrogen cyanide. The inflammable region then constitutes a band across the figure. Such a diagram can be drawn for various phosgene–air ratios. Since the effect of phosgene on both hydrogen cyanide and hexane is to bring the upper and lower limits closer together, the band becomes narrower with increasing values of the phosgene–air ratio, until it disappears at a value of the ratio equal to 0.247. No mixture of hydrogen cyanide, hexane, phosgene, and air would be inflammable in which the ratio of phosgene to air is greater than this value.

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A NOTE ON THE EFFECT OF CARBON DIOXIDE UPON THE INFLAMMABILITY LIMITS OF HYDROGEN CYANIDE[1]

By K. J. McCallum and Helen M. Trainor

Abstract

The inflammability limits of mixtures of carbon dioxide and hydrogen cyanide in air have been investigated. The effect of the addition of carbon dioxide is to bring the upper and lower inflammability limits of hydrogen cyanide together, until they meet at a composition containing 36 volume per cent carbon dioxide and 12 volume per cent hydrogen cyanide.

Introduction

In a previous paper (2) the effect of the vapors of cyanogen chloride, phosgene, chloroform, hexane, heptane, and methyl chloroformate upon the inflammability limits of hydrogen cyanide in air was reported. This note reports the effect of carbon dioxide upon these limits.

Materials and Procedure

Commercial grade liquid hydrogen cyanide was dried with anhydrous calcium chloride. Successive fractions of the vapor were condensed and analyzed by the method previously described. The experimental values found were 100.4% hydrogen cyanide on all fractions.

The carbon dioxide was obtained from the sublimation of dry ice. The gas was analyzed by absorbing a measured volume in potassium hydroxide solution. The residual unabsorbable gas was 0.1% by volume.

The gas mixtures were tested for inflammability in a Pyrex tube 47.5 mm. inside diameter and 103 cm. long. The procedure used in preparing the gas mixture was the same as that previously described (2). All observations were made with upward propagation of the flame and with the lower end of the tube open to the atmosphere. A small gas flame was used as the source of ignition.

Various mixtures of gases were tested until compositions were found that were approximately 0.5% on either side of a limit mixture.

Results and Discussion

In Table I are given the inflammability limits on mixtures of hydrogen cyanide, carbon dioxide, and air. In the first two columns are given the partial pressures of hydrogen cyanide and carbon dioxide between which the limits were found to lie. The compositions of the limit mixtures in volume per cent, given in the last three columns, were calculated on the assumption that partial pressure per cent is equal to volume per cent for these mixtures.

[1] *Manuscript received January 10, 1949.*

Contribution from the Department of Chemistry, University of Saskatchewan, Saskatoon, Saskatchewan.

TABLE I

INFLAMMABILITY LIMITS FOR HYDROGEN CYANIDE – CARBON DIOXIDE – AIR

Partial pressure of limit mixtures, mm. mercury		Barometer height, mm. mercury	Vol. % HCN (mean)	Vol. % CO_2 (mean)	Vol. % air (mean)
HCN	CO_2				
97.0– 97.5	242 –246	712.5	13.62	34.25	52.13
87.3– 87.8	255 –258	712.5	12.29	36.00	51.71
60.9– 65.2	71.8– 72.0	718.8	8.77	10.01	81.22
111 –115	225.7–226.4	717.2	15.76	31.52	52.72
84.6– 84.9	256.3–260.1	717.2	11.80	36.00	52.20
180.0–180.4	130.0–134.7	720.0	25.05	18.40	56.55
235.2–236.0	65.0– 68.7	723.8	32.50	9.25	58.25
91.1– 91.8	257.5–259.1	720.0	12.70	35.90	51.40
142.3–142.7	177.1–185.2	712.5	20.00	25.48	54.52
60.5– 64.6	141.5–142.0	712.5	8.76	19.88	71.36
78.7– 81.1	249.4–250.0	712.5	11.20	35.00	53.80
72.9– 76.0	216.8–217.5	723.4	10.28	30.00	59.72
75.3– 77.5	234.0–236.8	714.3	10.72	33.00	56.28

The plot of the results in Fig. 1 shows that the addition of carbon dioxide causes a rise in the lower inflammability limit and a decrease in the upper inflammability limit until the limits coincide, the maximum in the curve

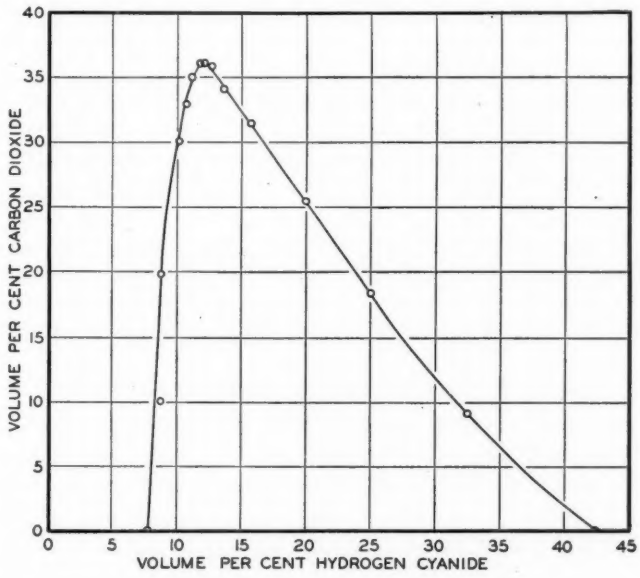

FIG. 1. *Inflammability limits in the system carbon dioxide – hydrogen cyanide – air.*

occurring at 36 volume % carbon dioxide and 12 volume % hydrogen cyanide. Although the maximum in the curve for the system hydrogen cyanide – cyanogen chloride – air occurs at nearly the same position (36 volume % cyanogen chloride and 12 volume % hydrogen cyanide) (2), the effect of the addition of cyanogen chloride differs from that of carbon dioxide in that small amounts of cyanogen chloride cause an initial decrease in the lower inflammability limit of hydrogen cyanide.

The volume per cents of added vapor at the maximum of the inflammability curves for hydrogen cyanide with added phosgene and chloroform are 15% and 16%, respectively (2), definitely lower than the values for added carbon dioxide and cyanogen chloride. These results are in qualitative agreement with the observation (1) that the heat capacity of the added vapor may be one factor in determining its effect upon the inflammability.

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RELATIVE REACTIVITIES OF THE GEOMETRICAL ISOMERS OF HEXACHLOROCYCLOHEXANE[1]

By D. J. Whittingham[2] and D. L. Garmaise

Abstract

The dehydrochlorination of hexachlorocyclohexane to trichlorobenzene in an alcoholic solution of piperidine was investigated, and the relative reactivities of four geometrical isomers of hexachlorocyclohexane determined. Activation energies and *PZ* values for the alpha, gamma, and delta isomers were obtained; the beta isomer reacted too slowly under the conditions used for convenient rate studies to be made. The order of reactivity was found to be alpha, delta > gamma ≫ beta; the order of reactivity is interpreted on the basis of differing degrees of steric hindrance in the isomers.

Introduction

The discovery in 1942 of the insecticidal properties of the gamma isomer of 1,2,3,4,5,6-hexachlorocyclohexane led to a renewed interest in the geometrical isomerism of this compound. It was first prepared in 1825 by Faraday (4) by bubbling chlorine into benzene in the sunlight; the existence of more than one isomer was recognized in 1884 by Schüpphaus (8) through observation of two crystal forms. Van der Linden (7) in 1912 succeeded in isolating four isomers, named alpha, beta, gamma, and delta, with widely different melting points and solubilities. Recently the isolation of a new isomer was reported by Kauer, DuVall, and Alquist (6). Hassel and Kringstad (5) have proposed that cyclohexane exists only in the chair, or "*Z*" form; if this is true for hexachlorocyclohexane, then eight geometrical isomers are theoretically possible, of which one is molecularly asymmetric.

Of the known isomers, it soon appeared evident that the beta isomer, the highest melting and most stable, had an alternating up and down arrangement of chlorines, which leads to the creation of an almost planar ring of chlorine atoms, known as the "beta ring", encircling the carbons (3). The other isomers then have one or more chlorines displaced outside of the beta ring.

The purpose of this work was to determine the relative ease of dehydrochlorination of the then known isomers (this work was undertaken before the isolation of the epsilon isomer) as an aid in proof of structure, and to test the proposal that insecticidal activity is associated with ease of dehydrochlorination.

Preparation and Separation of the Isomers

Chlorine was passed through distilled water and concentrated sulphuric acid, into a 1 liter flask containing 300 gm. of dry benzene, for 23 hr. at 20° C. The reaction mixture was irradiated by a mercury discharge lamp.

[1] *Manuscript received December 17, 1948.*
Contribution from the Chemical Laboratories of the University of New Brunswick, Fredericton, N.B.

[2] *Now at University College, London.*

The crude product (166 gm.) was then fractionally crystallized using a variety of solvents, following the procedure given by Smart (9). The amounts of isomers obtained pure were:

Alpha	102.0 gm., m.p. 157 to 157.5° C.
Beta	5.1 gm., m.p. 308 to 309° C.
Gamma	16.0 gm., m.p. 112 to 112.5° C.
Delta	2.9 gm., m.p. 138 to 139° C.

Melting points are corrected.

The reaction studied was the known conversion of hexachlorocyclohexane to a mixture of trichlorobenzenes using an alkaline reagent dissolved in alcohol. Preliminary trials indicated that potassium hydroxide caused too fast, and pyridine too slow, a reaction for convenient rate studies; the secondary amine, piperidine was adopted as the most convenient alkaline reagent.

The standard experimental procedure was to dissolve 0.3623 gm. (1.25×10^{-3} mole) of the hexachlorocyclohexane isomer in 95% ethyl alcohol and add 50.00 ml. of 0.0747 *N* alcoholic piperidine solution (3.74×10^{-3} mole), and to add further ethyl alcohol to bring the volume to 100.0 ml. The reaction solution was contained in an 8 in. test tube held by a stopper in a 1 liter three-necked flask, which served as a constant temperature vapor bath. Various solvents were refluxed in the flask to give the desired reaction temperatures. At intervals, 10.00 ml. aliquots were removed with a calibrated pipette, added to 50 ml. of water to quench the reaction, and the unreacted piperidine titrated with standard hydrochloric acid solution, using the mixed indicator bromcresol green – methyl red (3 : 2). The extent of reaction for a given time interval was calculated on the basis of the equation

$$C_6H_6Cl_6 + 3C_5H_{10}NH \longrightarrow C_6H_3Cl_3 + 3C_5H_{10}NH \cdot HCl$$

Fig. 1 shows the rate of dehydrochlorination of the alpha isomer at various temperatures.

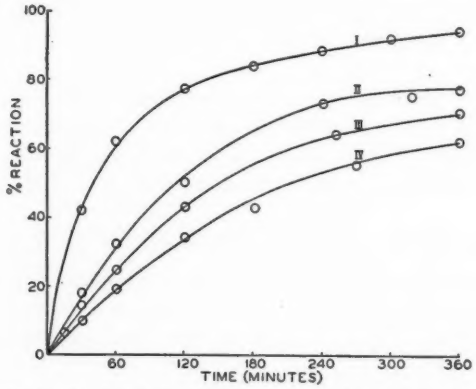

FIG. 1. *Dehydrochlorination of the alpha isomer at various temperatures. Initial concentration of alpha isomer = 0.0125 mole per liter.*
I, 79.5° C.; II, 65.0; III, 61.5; IV, 56.2.

Analogous families of curves were obtained with the gamma and delta isomers; with the beta isomer the rate was so slow under these conditions that good results could not be obtained. Only 15% of the beta isomer had reacted after seven days at 79.5° C.

The reaction was shown to follow a second-order equation for the alpha, gamma, and delta isomers; straight line relations between time of reaction and $\frac{x}{a-x}$ were obtained (Figs. 2, 3, and 4), where

x = moles of hexachlorocyclohexane isomer which react in time t,

a = moles of isomer originally present.

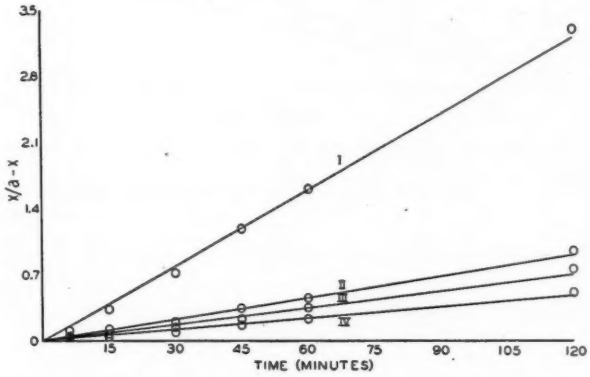

FIG. 2. *Determination of second-order rate constants for the alpha isomer at various temperatures. a = 0.0125 mole per liter.*
I, 79.5° C.; II, 65.0; III, 61.5; IV, 56.2.

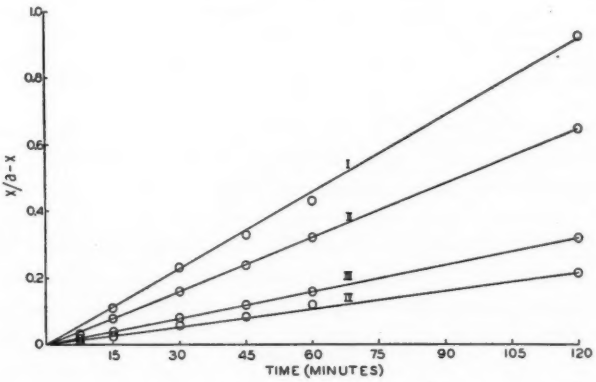

FIG. 3. *Determination of second-order rate constant for the gamma isomer at various temperatures. a = 0.0125 mole per liter.*
I, 79.5° C.; II, 72.0; III, 61.5; IV, 56.2.

The data on the rate of reaction of the beta isomer were inadequate for other than qualitative comparison.

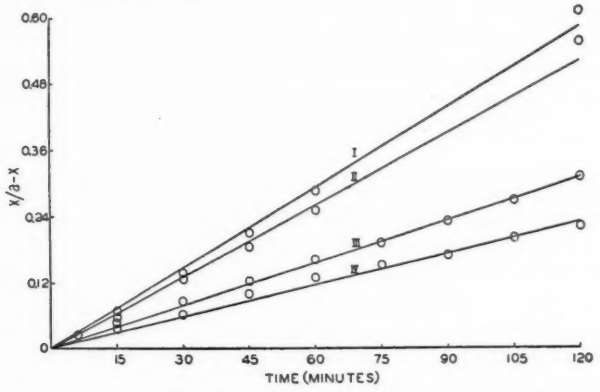

FIG. 4. *Determination of second-order rate constants for the delta isomer at various temperatures.*

I	*79.5° C.*	$a = 0.00230$ *mole per liter.*
II	*72.0*	$a = 0.00384$ *mole per liter.*
III	*61.5*	$a = 0.00767$ *mole per liter.*
IV	*56.2*	$a = 0.00767$ *mole per liter.*

The second-order rate constants obtained from the above data are listed in Table I.

TABLE I

SECOND-ORDER RATE CONSTANTS OF THE ISOMERS AT VARIOUS TEMPERATURES

Temperature, °C.	*k*, liters/mole/sec.		
	Alpha × 10^3	Gamma × 10^3	Delta × 10^3
79.5	12.5	3.4	11.9
72.0		2.6	6.4
65.0	3.8		
61.5	2.7	1.2	1.9
56.2	2.0	0.92	1.3

For the beta isomer; at 79.5° C., "k" is roughly 10^{-6}.

These values of k were plotted in the graph log k against $\frac{1}{T}$ (Fig. 5) to obtain activation energies and the values of the PZ factor in the Arrhenius equation. From this graph, the values for E and PZ listed in Table II were obtained.

Discussion

The great resistance to dehydrohalogenation of the beta isomer, as evidenced by its extremely low rate of reaction in comparison with the other isomers, appears to justify the concept of the "beta ring" of chlorines, in which, it is

presumed, there would be a high degree of steric hindrance. The order of reactivities of the other isomers, alpha, delta > gamma ≫ beta, coincides with the proposed structures arising from the statistical treatment of the

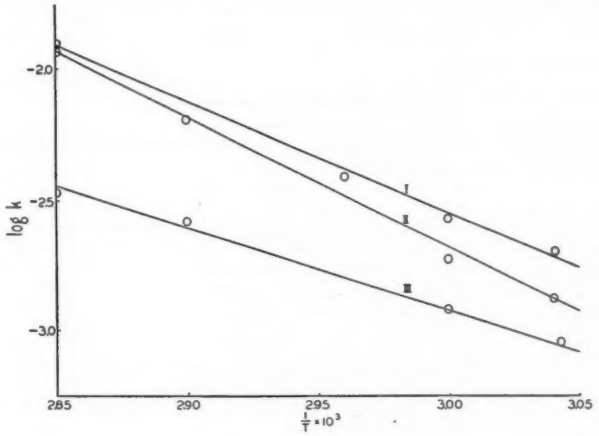

FIG. 5. *Relation of log k to* $\frac{1}{T}$ *for the alpha, gamma, and delta isomers. I, alpha; II, delta; III, gamma.*

TABLE II

ACTIVATION ENERGIES AND *PZ* VALUES FOR THE ISOMERS

Isomer	*E*, kilocalories	*PZ*
Alpha	19.5	2×10^{10}
Gamma	13.8	1×10^{6}
Delta	20.4	6×10^{10}

likelihood of formation of each isomer (1). These studies, although they are probably not conclusive, led to the assumption that the gamma isomer has one chlorine out of the beta ring, and the alpha and delta isomers each two. It is interesting to note that the active gamma isomer shows an intermediate reactivity, which indicates that the process of insecticidal action probably does not involve dehydrohalogenation. The proposed structure of the gamma isomer is sterically identical with the proven structure of the metabolite mesoinositol. The latter compound, hexahydroxycyclohexane, has several known geometrical isomers, all relatively unimportant physiologically. Attempts made in this laboratory to interconvert the active isomers of hexachlorocyclohexane and inositol, by hydrolysis or chlorination, have been unsuccessful.

Subsequent to the undertaking of this work, studies on the rate of dehydrochlorination of the hexachlorocyclohexane isomers were reported by Cristol (2) and by Kauer *et al.* (6), the latter including the epsilon isomer in the investigation. These workers used alcoholic sodium hydroxide rather than piperidine as the dehydrochlorination agent, but the experimental results are essentially in agreement. The epsilon isomer was reported to be somewhat less reactive than the gamma isomer.

Acknowledgment

Acknowledgment is made to the Defence Research Board for awarding a grant in aid of research on this project.

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SYNTHÈSES D'ACIDES AMINÉS PAR L'INTERMÉDIAIRE DES HYDANTOÏNES. DL-SÉRINE ET DL-CYSTINE[1]

PAR GUY NADEAU ET ROGER GAUDRY

Résumé

Le méthoxyacétal obtenu du bromacétal avec un rendement de 78% fut transformé en aldéhyde, puis en cyanhydrine correspondante. La cyanhydrine, chauffée avec un excès de carbonate d'ammonium suivant la modification de Bucherer de la méthode de Strecker, donna la 5-méthoxyméthylhydantoïne avec un rendement de 52%. L'hydrolyse de l'hydantoïne avec de l'hydroxyde de baryum à 160° C. sous pression donna l'acide α-amino-β-méthoxypropionique qui fut hydrolysé en DL-sérine par la méthode usuelle. La réaction de Bucherer appliquée à l'éthoxyacétal, au chloracétal, au bromacétal, au méthylthioacétal et au benzylthioacétal donna la 5-éthoxyméthylhydantoïne, la 5-chlorométhylhydantoïne, la 5-bromométhylhydantoïne, la 5-méthylthiométhylhydantoïne et la 5-benzylthiométhylhydantoïne avec des rendements respectifs de 36%, 63%, 29%, 28% et 76%. L'hydrolyse de la 5-méthylthiométhylhydantoïne et de la 5-benzylthiométhylhydantoïne au moyen de l'hydroxyde de baryum à 160° C. sous pression donna la S-méthylcystéine et la S-benzylcystéine avec des rendements respectifs de 75% et 68%. L'hydrolyse de la 5-chlorométhylhydantoïne avec de l'acide chlorhydrique à 20% sous pression à 130° C. donna 56% d'acide α-amino-β-chloropropionique que l'hydroxyde d'argent permet de transformer en DL-sérine.

Introduction

La première synthèse de la DL-sérine par la méthode de Strecker (16) a été faite par Fischer et Leuchs (8) à partir de l'aldéhyde glycolique. A cause de l'instabilité relative de ce produit de départ, Leuchs et Geiger (12) lui substituèrent l'éthoxyacétaldéhyde qu'ils obtinrent du chloracétal. Plus récemment Dunn *et al.* (5) préparèrent l'éthoxyacétaldéhyde par oxydation de l'éther monoéthylique du glycol d'éthylène, produit commercial facilement accessible, et décrivirent la première méthode pratique de synthèse de la sérine par la méthode de Strecker. Mais la sérine n'avait jamais encore été préparée en passant par les hydantoïnes substituées en position 5 d'après la modification de Bucherer (3) de la méthode classique de Strecker.

Nous avons préparé la 5-méthoxyméthylhydantoïne ainsi que la 5-éthoxyméthylhydantoïne à partir du chloracétal et du bromacétal par l'intermédiaire du méthoxyacétal et de l'éthoxyacétal. Leuchs et Geiger (12) avaient eux-mêmes utilisé le chloracétal dans la préparation de l'éthoxyacétal d'après la méthode décrite par Lieben (14) tandis que Pinner (15) et Levene et Schormuller (13) lui avaient substitué le bromacétal. Nous avons étudié la possibilité de remplacer l'éthoxyacétal par le méthoxyacétal. En simplifiant la méthode de Leuchs et Geiger, nous avons obtenu le méthoxyacétal avec un rendement de 71% et l'éthoxyacétal avec un rendement de 63% à partir

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Contribution du Département de biochimie de la Faculté de médecine, Université Laval, Québec, Canada. Extrait de la thèse présentée par Guy Nadeau à l'Ecole des gradués de l'Université Laval pour l'obtention du grade de docteur ès sciences physiques.

du chloracétal. Toutefois le fractionnement du méthoxyacétal ou de l'éthoxyacétal en présence de chloracétal non transformé est difficile et, comme l'avaient d'ailleurs indiqué Leuchs et Geiger, il est pratiquement impossible d'éviter la présence de chlore dans les produits des réactions subséquentes.

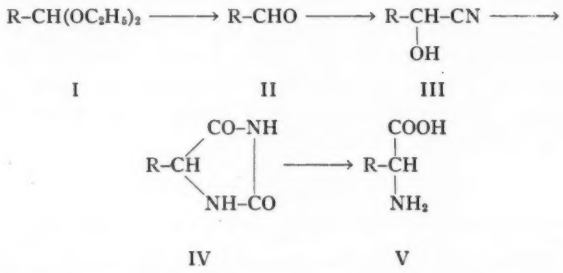
Afin de parer à cet inconvénient, nous avons alors substitué au chloracétal le bromacétal que nous avons préparé à partir de l'acétate de vinyle suivant la méthode de Bedoukian (2), mais avec un rendement de 87.5%. Nous avons ainsi porté le rendement en méthoxyacétal à 78% et en éthoxyacétal à 73.5%. Cette modification a en outre l'avantage de donner des produits exempts de brome à cause de la facilité de séparer par fractionnement le bromacétal non transformé.

Pour hydrolyser l'acétal (I) en aldéhyde correspondante (II) (6, 11, 12), nous avons trouvé que les meilleures conditions consistaient à traiter l'acétal par un acide minéral 5 *N* et à agiter le tout efficacement à la température de la chambre. Sans qu'il soit nécessaire d'isoler l'aldéhyde de sa solution aqueuse, nous en avons préparé le dérivé bisulfitique, puis la cyanhydrine (III) qui fut extraite à l'éther de la solution aqueuse. Le chauffage de la cyanhydrine avec un excès de carbonate d'ammonium (3) a donné la 5-méthoxyméthylhydantoïne avec un rendement de 52% à partir du méthoxyacétal et la 5-éthoxyméthylhydantoïne avec un rendement de 36% à partir de l'éthoxyacétal. Il est à noter toutefois que la 5-méthoxyméthylhydantoïne est beaucoup plus facile à isoler à l'état pur et à recristalliser que le composé éthoxylé et, comme le méthoxyacétal s'obtient lui-même avec un meilleur rendement, il n'y a donc pas d'avantage à effectuer cette synthèse de la sérine par l'intermédiaire du dérivé éthoxylé.

Nous avons hydrolysé la 5-méthoxyméthylhydantoïne au moyen de l'hydroxyde de baryum en acide α-amino-β-méthoxypropionique, composé difficilement cristallisable, mais que nous avons identifié sous forme d'acide α(β-phényluréido)-β-méthoxypropionique. L'hydrolyse de cet acide aminé suivant la méthode décrite par Carter et West (4, p. 82) a donné la DL-sérine avec un rendement de 72.4% à partir de la 5-méthoxyméthylhydantoïne, soit un rendement de 37.7% à partir du méthoxyacétal.

Nous avons aussi appliqué au chloracétal et au bromacétal la réaction de Bucherer (3) et nous avons obtenu la 5-chlorométhylhydantoïne et la 5-bromométhylhydantoïne avec des rendements respectifs de 63% et 29%. L'instabilité plus grande de l'hydantoïne bromée en milieu alcalin explique le faible rendement obtenu comparativement à l'hydantoïne chlorée. Pour la même raison, nous avons effectué l'hydrolyse de la 5-chlorométhylhydantoïne en acide DL-α-amino-β-chloropropionique au moyen de l'acide chlorhydrique à 20% sous pression, avec un rendement de 56%. A notre connaissance, la synthèse directe de cet acide aminé n'avait pas été réalisée auparavant, mais il avait été préparé à partir de la sérine par Fischer et Raske (9) et Erlenmeyer et Stoop (7) comme intermédiaire au cours de leur synthèse de la cystine.

Nous avons ensuite transformé l'acide DL-α-amino-β-chloropropionique en DL-sérine au moyen de l'hydroxyde d'argent avec un rendement de 87%, soit 31% à partir du chloracétal.



R = méthoxyméthyl, (CH_3–O–CH_2–); éthoxyméthyl, (CH_3–CH_2–O–CH_2–); chlorométhyl, (Cl–CH_2–); bromométhyl, (Br–CH_2–); méthylthiométhyl, (CH_3–S–CH_2–); benzylthiométhyl, (C_6H_5–CH_2–S–CH_2–).

Le passage de la 5-chlorométhylhydantoïne ou de la 5-bromométhylhydantoïne à la 5-méthoxy- ou à la 5-éthoxyméthylhydantoïne s'est avéré impossible à cause de l'insolubilité de ces hydantoïnes halogénées dans l'alcool absolu. Toutefois nous avons préparé l'hydantoïne correspondante de la sérine, la 5-hydroxyméthylhydantoïne, à partir de la 5-chlorométhylhydantoïne au moyen de l'hydroxyde d'argent.

Nous avons en outre préparé la 5-méthylthiométhylhydantoïne et la 5-benzylthiométhylhydantoïne avec des rendements respectifs de 28.4% et 76.5% à partir du méthylthioacétal et du benzylthioacétal. Ces acétals avaient déjà été préparés respectivement par Barger et Coyne (1) et Hutchison et Smiles (10). Toutefois, en simplifiant leurs méthodes et en substituant le bromacétal au chloracétal, nous avons porté les rendements en méthylthioacétal et en benzylthioacétal à 88.5% et 80% respectivement. L'hydrolyse des hydantoïnes avec de l'hydroxyde de baryum à 160° C. sous pression nous a donné la S-méthylcystéine et la S-benzylcystéine avec des rendements de 75% et 68%.

A notre connaissance, la synthèse directe de la S-méthylcystéine n'avait pas été décrite antérieurement. Cet acide aminé avait été préparé par méthylation de la DL-cystéine par duVigneaud, Loring et Craft (17). La S-benzylcystéine avait été préparée par Wood et duVigneaud (18), à partir du sulfure de chlorométhylbenzyle par condensation avec le phtalimidomalonate d'éthyle et hydrolyse, comme intermédiaire dans leur synthèse de la DL-cystine.

Le rendement total en DL-cystine à partir du bromacétal est donc de 33% puisque l'hydrolyse de la S-benzylcystéine au moyen du sodium dans l'ammoniac liquide se fait avec un rendement de 80%.

Partie expérimentale

Alkoxyacétals (I)

Un mélange de bromacétal (0.2 mole) et d'une solution de méthylate ou d'éthylate de soude (0.2 mole de sodium dans 100 ml. de méthanol ou d'éthanol absolus) fut chauffé dans un autoclave à 105°-110° C. pendant une heure, puis refroidi et versé dans 500 ml. d'eau froide. L'acétal fut extrait à l'éther et la solution éthérée fut séchée sur du sulfate de soude. Après distillation de l'éther, l'acétal fut distillé dans le vide. Le tableau I décrit les propriétés et les rendements de ces acétals.

TABLEAU I

ACÉTALS

Acétal	Produit de départ	Rendement, %	P.é., ° C.
Méthoxyacétal	Chloracétal	71	48–50 (19 mm.)
"	Bromacétal	78	
Éthoxyacétal	Chloracétal	63	71–72 (25 mm.)*
"	Bromacétal	73.5	
Méthylthioacétal	Bromacétal	88.5	91 (25 mm.)†
Benzylthioacétal	Bromacétal	80	178 (25 mm.)‡

* *72° à 74° C. (26 mm.) (12).*
† *188° à 190° C. (760 mm.) (1).*
‡ *192° à 195° C. (30 mm.) (10).*

Thioacétals (I)

A une solution d'éthylate de soude (0.2 mole de sodium dans 200 ml. d'éthanol absolu), refroidie dans un bain de glace et de sel, furent ajoutés lentement le méthyl ou le benzyl mercaptan (0.2 mole) liquide, préalablement refroidi, puis le bromacétal (0.2 mole). Le mélange fut chauffé à reflux pendant deux heures, refroidi et versé dans 500 ml. d'eau froide. L'acétal fut extrait à l'éther et la solution éthérée fut séchée sur du sulfate de soude. Après distillation de l'éther, l'acétal fut distillé dans le vide. Le tableau I décrit les propriétés et les rendements de ces acétals.

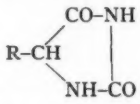
Hydantoïnes (IV)

Un mélange de l'acétal (0.1 mole) et de 20 ml. d'acide chlorhydrique 5 *N* fut agité à la température de la chambre pendant 24 h. A la solution refroidie et agitée furent ajoutés successivement du sulfite neutre de soude (0.1 mole), du sulfite acide de soude (0.1 mole) et une solution de cyanure de potassium (0.1 mole) dans 50 ml. d'eau. Le mélange fut agité pendant deux heures à la température de la chambre. Après extraction à l'éther et distillation de l'éther dans un vide partiel, la cyanhydrine résultante fut ajoutée à un mélange de carbonate d'ammonium (0.2 mole) et de 100 ml. d'alcool à 50%. Le mélange fut agité pendant deux heures dans un bain-marie maintenu à 55° C. et l'excès de carbonate d'ammonium fut décomposé par distillation

dans le vide. La solution fut décolorée au noir animal, concentrée à petit volume et additionnée de deux ou trois volumes d'alcool bouillant. L'hydantoïne cristallise lentement à la glacière. Le tableau II décrit les propriétés et les rendements des hydantoïnes.

TABLEAU II

HYDANTOÏNES



R-	Rendement, %	P.f., ° C.	Formule	Analyse, %			
				Azote		Soufre ou halogène	
				Calculé	Trouvé	Calculé	Trouvé
5-méthoxyméthyl-	52.1	167–170 (sub.)*	$C_5H_8O_3N_2$	19.44	19.37	—	—
5-éthoxyméthyl-	36.1	77	$C_6H_{10}O_3N_2$	17.72	17.48	—	—
5-chlorométhyl-	63.2	170 (déc.)	$C_4H_5O_2N_2Cl$	18.85	18.99	23.88	23.90
5-bromométhyl-	28.7	165 (sub.)	$C_4H_5O_2N_2Br$	14.51	14.28	41.45	40.82
5-méthylthiométhyl-	28.4	154–155	$C_5H_8O_2N_2S$	17.50	17.50	20.00	18.85
5-benzylthiométhyl-	76.5	103–106	$C_{11}H_{12}O_2N_2S$	10.86	10.92	13.56	13.08

* *Les points de fusion ne sont pas corrigés.*

La cristallisation de la 5-chlorométhylhydantoïne se fait par évaporation de la solution aqueuse. Dans ce cas, l'addition d'alcool donne un précipité amorphe, non recristallisable. D'autre part, la 5-benzylthiométhylhydantoïne cristallise instantanément par agitation du résidu sec avec de l'éther de pétrole.

DL-Sérine

Un mélange de 7.2 g. de 5-méthoxyméthylhydantoïne, de 25.2 g. d'hydroxyde de baryum octahydraté et de 150 ml. d'eau fut chauffé dans un autoclave à 160° C. pendant 30 min. Le mélange fut refroidi et le carbonate de baryum formé fut filtré. Le filtrat fut agité avec 4.5 g. de carbonate d'ammonium et filtré de nouveau. L'excès de carbonate d'ammonium fut décomposé par distillation dans le vide et la solution fut évaporée à siccité. Le résidu brut de l'O-méthylsérine fut chauffé à reflux avec 40 ml. d'acide bromhydrique à 48% pendant deux heures et demie. La DL-sérine fut isolée suivant la méthode décrite par Carter et West (4). P.f. 240° C. (d.). Rendement: 3.8 g., 72.4%. Calculé pour $C_3H_7O_3N$: N, 13.33%. Trouvé: 13.28%.

Acide α-Amino-β-chloropropionique

Un mélange de 3.0 g. de 5-chlorométhylhydantoïne et de 40 ml. d'acide chlorhydrique à 20% dans un tube scellé à vide fut chauffé pendant 36 h. à

125°-130° C. La solution fut évaporée à siccité et le chlorhydrate de l'acide aminé fut séparé du chlorure d'ammonium par dissolution dans l'alcool absolu bouillant. La solution fut additionnée d'un léger excès de pyridine et mise à la glacière. L'acide aminé brut fut recueilli par filtration, dissous dans l'eau, décoloré au noir animal et recristallisé par addition de deux volumes d'alcool bouillant. Le tableau III décrit les propriétés et le rendement de l'acide aminé.

TABLEAU III

ACIDES AMINES OBTENUS À PARTIR DES HYDANTOÏNES CORRESPONDANTES

$$R\text{–}\underset{\displaystyle NH_2}{\underset{|}{CH}}\text{–}COOH$$

Acide aminé	Rendement, %	P.f., ° C.	Formule	Analyse, %			
				Azote		Soufre ou halogène	
				Calculé	Trouvé	Calculé	Trouvé
O-méthylsérine*	80	—	—	—	—	—	—
Acide α-amino-β-chloropropionique	56	159–160(sub.)†	$C_3H_6O_2NCl$	11.33	11.20	28.74	28.43
S-méthylcystéine	75	240 (déc.)‡	$C_4H_9O_2NS$	10.37	10.22	23.70	22.88
S-benzylcystéine	68	215§	$C_{10}H_{13}O_2NS$	6.63	6.65	15.16	13.08

* *Dérivé: acide α (β-phényluréido)-β-méthoxypropionique. P.f. 164° à 166° C. Calculé pour* $C_{11}H_{14}O_4N_2$: *N, 11.76%. Trouvé: N, 11.53%.*

† *Vers 160° C. (9).*

‡ *248° C. (déc.) (17)*

§ *215° à 216° C. (18).*

DL-Sérine

Une solution de 2.0 g. d'acide α-amino-β-chloropropionique dans 50 ml. d'eau fut agitée pendant une heure à la température de la chambre avec 2 g. d'hydroxyde d'argent fraîchement préparé. Le mélange fut filtré et le filtrat fut débarrassé des sels d'argent par l'hydrogène sulfuré. Après filtration, la solution fut évaporée à siccité et le résidu fut dissous dans 10 ml. d'eau. A la solution refroidie à 0° C. et fortement agitée furent ajoutés 10 ml. d'éthanol absolu. L'agitation fut poursuivie pendant une heure à 0° C. puis le mélange fut mis à la glacière. La DL-sérine cristallise lentement. Rendement: 1.45 g., 86.8%. P.f. 240° C. (d.). Calculé pour $C_3H_7O_3N$: N, 13.33%. Trouvé: N, 13.20%.

5-Hydroxyméthylhydantoïne

Un mélange de 7.4 g. de 5-chlorométhylhydantoïne en solution dans 200 ml. d'eau et de 6.5 g. d'hydroxyde d'argent fraîchement préparé fut agité à la température de la chambre pendant 30 min. Le précipité formé fut séparé par centrifugation et les dernières traces de sel d'argent furent précipitées

par l'hydrogène sulfuré. La solution résultante fut décolorée au noir animal et évaporée à siccité. Le résidu fut cristallisé par agitation avec 50 ml. d'alcool absolu. P.f. 140° C.(sublime). Calculé pour $C_4H_6O_3N_2$: N, 21.54%. Trouvé: N, 21.49%.

S-méthyl- et S-benzylcystéine

Un mélange de l'hydantoïne (0.05 mole), de 25.2 g. d'hydroxyde de baryum octahydraté et de 150 ml. d'eau fut chauffé dans un autoclave à 160° C. pendant 30 min. Le mélange fut agité avec 4.5 g. de carbonate d'ammonium et la carbonate de baryum filtré. L'excès de carbonate d'ammonium fut décomposé par distillation dans le vide. La solution fut décolorée au noir animal, concentrée à petit volume, additionnée de trois volumes d'alcool bouillant et mise à la glacière. Le tableau III décrit les propriétés et les rendements des acides aminés.

Remerciements

Les auteurs remercient le Conseil National des Recherches pour l'octroi dont ils ont bénificié au cours de ce travail.

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INFLUENCE OF THE AMINO ACID – DEXTROSE REACTION ON GROWTH OF LACTIC ACID BACTERIA[1]

By Dyson Rose and Ruth Peterson

Abstract

Growth of *Lactobacillus arabinosus, L. casei,* and *Streptococcus faecalis* (as measured by lactic acid production) was studied in relation to the effects of the products of the amino acid – reducing sugar (Maillard) reaction. Addition of preformed Maillard products to a medium had little or no effect. Medium that had been autoclaved after the addition of dextrose promoted more rapid growth (shorter lag phase) than medium for which the dextrose had been autoclaved separately. This effect could not be traced to the presence of Maillard products, but appeared to be a complex phenomenon depending in part on the *Eh* of the solution. Destruction of amino nitrogen occurred during autoclaving, and destruction of tryptophan was evident from a comparison of growth response curves. It is concluded that the Maillard reaction affects the growth of these organisms only when an essential amino acid (or other nutrient), present in limiting quantities, is destroyed by the reaction. A serious error may be introduced into microbiological assays for amino acids if the samples to be assayed contain dextrose.

Introduction

Dextrose-containing media for the culture of micro-organisms darken in color when autoclaved. The darkening is a function of time and temperature, and, when it is intense, growth of some organisms is frequently less vigorous. Recent studies have led to recognition of much of the darkening of media during heat sterilization as an example of the amino acid – reducing sugar (Maillard (4)) reaction. It appeared possible, therefore, that products formed by the reaction of amino acids with dextrose might have inhibitory effects on the growth of some species of bacteria, and the present work was undertaken to determine the extent to which this inhibition might affect species commonly used in microbiological assays.

Stanier (11) presented evidence to show that heat sterilization of glucose in the presence of mineral salts rendered it toxic to *Cytophaga*, but Fahraeus (1) was unable to confirm this finding even for the same species. Hill and Patton (3) showed that the growth response of *Streptococcus faecalis* to given trytophan levels was lowered if dextrose was heat sterilized with the other constituents of the medium but concluded (7) that this was due to a destruction of tryptophan.

The studies with *Cytophaga* (1, 11) were conducted using a medium containing no protein or amino acid nitrogen, and which, therefore, could not have contained Maillard products. In studies requiring more complex media, two types of products may be present after heat sterilization (true carameliza-

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tion products and the nitrogen-containing products of the Maillard reaction) and it would be desirable to distinguish between them but, to our knowledge, no method is available.

Materials and Methods

Three organisms commonly used for vitamin and amino acid assays were chosen: *Lactobacillus arabinosus* (8014),* *L. casei* (7569),* and *S. faecalis* (9790).* Transfers from stab cultures to a complete liquid medium were made 24 hr. before test solutions were to be inoculated. Inoculum was prepared by centrifuging the organisms from the liquid medium, resuspending them in 2 ml. of 1% saline, and diluting four drops of this suspension with approximately 20 ml. of saline. One drop of dilute suspension was used to inoculate 10 ml. of culture medium.

Two types of media were used in the studies: the synthetic medium used for amino acid assays (similar to that of Stokes *et al.* (12)), and the casein hydrolyzate medium used for niacin or tryptophan assay (similar to that of Greene and Black (2) and Snell and Wright (10)). In some tests, dextrose was added before autoclaving so that Maillard product was formed *in situ*, in others the medium was prepared in the normal manner except that no dextrose was added; then 5 ml. of a 2% solution of dextrose, containing Maillard product if desired, was added to each tube of medium after autoclaving (total volume, 10 ml.). In a few experiments medium containing dextrose was sterilized by Seitz filtration.

Maillard product was formed for subsequent addition to a medium by subjecting a mixture of dextrose and casein hydrolyzate (General Biochemicals Ltd.) to a temperature of 140° F. for several days. In the absence of a suitable method for determining the type of reaction (caramelization or Maillard) that had occurred, all fluorescent compounds formed in the presence of amino acids were assumed to be Maillard products. The concentration of these compounds in the media was estimated by determining the relative fluorescence with a Coleman Model 12 photofluorometer and B_1 and PC_1 filters.

The extent of growth of the organisms was determined by titrating the 10 ml. of culture medium with 0.1 *N* sodium hydroxide and the results are presented as milliliters of sodium hydroxide used.

Results

Effect of Various Factors on Initial Growth of the Organisms

The rate of growth of these organisms was compared in medium prepared and autoclaved in the normal manner, medium to which sterile dextrose solution was added after autoclaving, and medium that had been sterilized by Seitz filtration.

* *American Type Culture Collection Numbers.*

The results are given in Figs. 1 and 2, and show that, in contrast to the normally autoclaved medium, little or no growth of *L. arabinosus* or of *L. casei* occurred during the first 24 hr. in medium for which the dextrose was

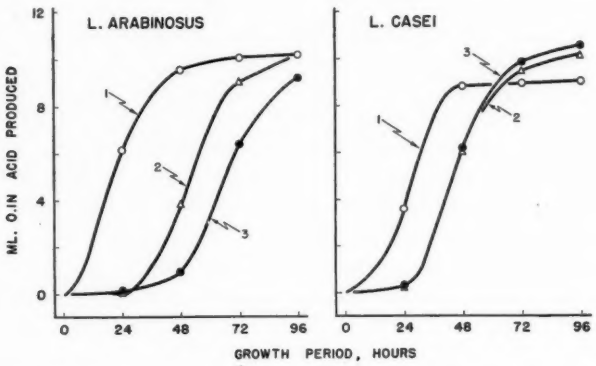

FIG. 1. *Effect of autoclaving amino acid assay medium on acid production.*
1. Medium autoclaved with dextrose, fluorescence 27.5.
2. Medium Seitz filtered, fluorescence 9.0.
3. Dextrose autoclaved separately, fluorescence 9.0.

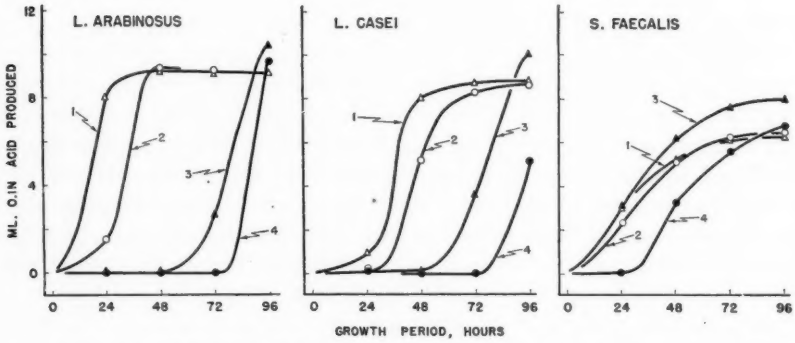

FIG. 2. *Effect of the weight of inoculum on acid production in autoclaved and unautoclaved amino acid assay medium.*
1. Medium autoclaved with dextrose, fluorescence 64, heavy inoculum.
2. Medium autoclaved with dextrose, fluorescence 64, light inoculum.
3. Dextrose autoclaved separately, fluorescence 9.0, heavy inoculum.
4. Dextrose autoclaved separately, fluorescence 9.0, light inoculum.

autoclaved separately. In a medium sterilized by Seitz filtration, a similar but less pronounced extension of the lag or induction period was observed. This prolongation of the lag phase was most marked if a light inoculum was used but was evident even in normally inoculated cultures (Fig. 2). *S. faecalis* was much less sensitive to factors inducing the prolonged lag phase than

were the two species of *Lactobacilli;* nevertheless, when a light *S. faecalis* inoculum was used, a slight but distinct prolongation of the lag phase occurred.

Attempts were made to duplicate this stimulatory effect of autoclaving by adding preformed Maillard products. Control tubes were autoclaved after the addition of dextrose; the others received, after autoclaving, a separately sterilized solution of dextrose that contained widely varying amounts of Maillard product. The results were not entirely consistent but indicated that initial growth was not influenced by the mere presence of Maillard product but depended upon some secondary factor introduced when the reaction occurred in the medium.

Results obtained when the initial pH of the medium was carefully controlled are presented in Fig. 3. These data show that the effect of autoclaving on the pH was not a critical factor.

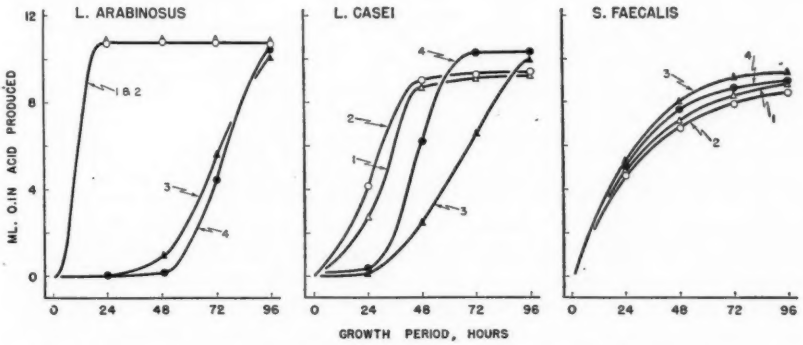

FIG. 3. *Effect of autoclaving amino acid medium, and of pH, on acid production.*
1. Medium autoclaved with dextrose, initial pH 6.78, final pH 6.51, fluorescence 26.
2. Medium autoclaved with dextrose, initial pH 6.51, final 6.32, fluorescence 22.
3. Dextrose autoclaved separately, initial pH 6.78, final 6.78, fluorescence 9.5.
4. Dextrose autoclaved separately, initial pH 6.51, final 6.52, fluorescence 9.5.

To change the oxidation–reduction potential of the medium a solution of ascorbic acid (20 mgm. in 250 ml.) and dextrose, sterilized by Seitz filtration, was added to one lot of medium before autoclaving. Growth in this lot, as shown in Fig. 4, greatly exceeded that in the tubes for which dextrose had been autoclaved separately and, with two organisms, exceeded that in normally autoclaved medium.

Determination of the *Eh* of these complex solutions gave results of doubtful significance. While the *Eh* of medium containing ascorbic acid was invariably negative (-0.03 to -0.04 v.), that of normal medium varied from $+0.02$ to $+0.08$ v. Medium autoclaved with dextrose tended to give lower values than that for which the dextrose had been autoclaved separately, but medium with added Maillard product sometimes gave still lower *Eh*

values. Within this range (+ 0.02 to + 0.08 v.) no correlation between the early growth rate of the organisms (24 hr. titer) and the *Eh* could be found.

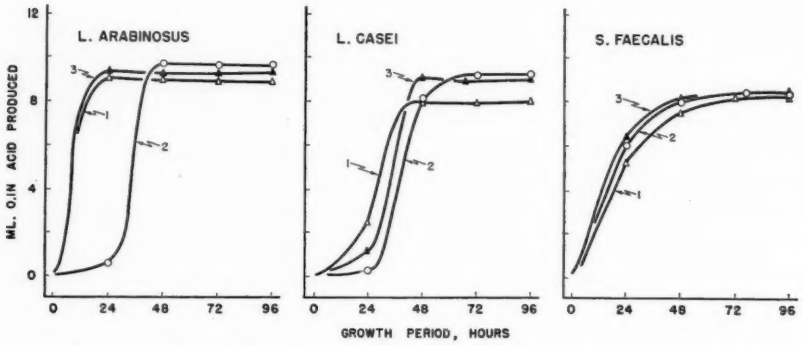

FIG. 4. *Effect of autoclaving tryptophan assay medium, and of ascorbic acid, on acid production.*

1. Medium autoclaved with dextrose, fluorescence 48.
2. Dextrose autoclaved separately, fluorescence 16.5.
3. Ascorbic acid added to medium, fluorescence 14.5.

Effect of Various Factors on Total Growth of the Organisms

When the dextrose is autoclaved with other constituents of the medium, reactions occur that destroy some dextrose. When Maillard products are formed externally to the medium a variable amount of dextrose usually remains unaffected by the reaction and is added to the medium with the Maillard products. This could be avoided by using the insoluble type of Maillard product but such material is not typical of that formed in media under the usual conditions of autoclaving. It was thus impossible to vary the amount of Maillard product in the medium without varying the sugar content but, in spite of this difficulty, attempts were made to determine the effect of Maillard product on total growth of the organisms.

For this purpose, varying amounts of a solution of Maillard products were added to weighed quantities of dextrose and the total volume made to 100 ml. After autoclaving, these solutions were dispensed to tubes of dextrose-free medium. For Expt. I of Table I the amount of dextrose was varied so that the total amount present, if none had been destroyed in the Maillard reaction, would have been 2 gm. per 100 ml. For Expts. II and III, 2 gm. of dextrose was used in each solution and the original dextrose content has been calculated to include that of the dextrose–casein hydrolyzate mixture.

The data presented in Table I show that total acid production by the two *Lactobacilli* paralleled the original dextrose content, and decreased with increasing fluorescence only in Expt. I in which no dextrose was added to offset that destroyed by the Maillard reaction. The total acid production by

TABLE I

EFFECT OF MAILLARD PRODUCT AND DEXTROSE CONCENTRATION ON THE TOTAL ACID PRODUCTION

Ml. of Maillard solution	Original dextrose, %*	Fluorescence	Ml. of 0.1 *N* acid produced in 120 hr.		
			L. arabinosus	*L. casei*	*S. faecalis*
Expt. I					
0	2.00	7	9.64	10.72	—
2.5	2.00	165	9.75	9.40	—
5.0	2.00	300	8.60	8.20	—
7.5	2.00	415	7.66	7.28	—
10.0	2.00	500	6.43	6.11	—
Expt. II					
0	2.00	11	10.54	10.42	7.59
2.5	2.23	780	11.31	11.27	7.91
5.0	2.45	2250	12.02	12.03	7.98
7.5	2.63	3100	13.07	12.83	8.03
Expt. III					
0	2.00	10	10.44	10.09	8.65
1	2.09	310	10.68	10.30	8.35
2	2.18	560	11.19	10.67	8.31
3	2.27	800	11.26	10.97	8.00

* *No. 1 of each experiment contained exactly the dextrose content shown; in the remainder, some dextrose had been destroyed in the formation of Maillard products.*

S. faecalis was less influenced by the original dextrose content of the medium and there is some evidence (Expt. III) of a mild toxicity. Even for this species, however, the toxicity is obviously very slight.

To confirm the influence of the available dextrose on total acid production, solutions of dextrose were prepared so that 5 ml. would supply 100, 110, 120, or 130 mgm. per tube. Tubes receiving these amounts were autoclaved after the addition of the dextrose, and other tubes were given exactly 100 mgm. after autoclaving. The results are presented graphically in Fig. 5*A* and indicate that, with both *L. arabinosus* and *L. casei*, acid production from 100 mgm. of dextrose added after autoclaving was equal to that from approximately 107.5 mgm. added before autoclaving. Many of the compounds in the medium, including the Maillard products, interfere in chemical methods of dextrose determination, and the latter are therefore not sufficiently accurate to corroborate this figure.

Similar experiments using *S. faecalis* did not show a linear relation between total acid production and dextrose content. This is believed to have been due to the low buffering capacity of the medium used.

Loss of an Essential Amino Acid from Media During Autoclaving

Formation of Maillard product in a medium involves the destruction of both dextrose and an amino acid. If the amino acid destroyed is essential, and is present in limiting quantities, its loss will reduce the growth of the

organism. The adequacy of autoclaved media used in these experiments, and of a "complete" yeast extract medium, was demonstrated by determining the growth response to increasing concentrations of dextrose. Data for *L.*

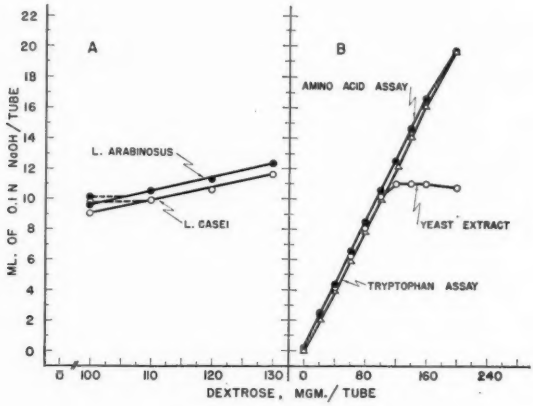

FIG. 5.

A. Effect of dextrose concentration on the total acid production by L. arabinosus and L. casei.

B. Effect of dextrose concentration on the total acid production by L. arabinosus in three types of media.

arabinosus are presented in Fig. 5*B*, and show that a direct linear response is obtained over a wide range of dextrose concentrations. With yeast extract medium some constituent other than dextrose obviously became limiting after the acid production reached 10.5 ml. *L. casei* behaved in a similar manner except that yeast extract medium was approximately equal to the others.

Total amino-nitrogen determinations, made by the micro Van Slyke manometric technique, showed that a considerable destruction of amino acids occurred during heat sterilization of tryptophan assay medium (Table II). Higher concentrations of dextrose led to a greater loss of amino nitrogen.

TABLE II

EFFECT OF AUTOCLAVING ON THE AMINO-NITROGEN CONTENT OF MEDIA

Treatment	Dextrose content, %	Amino-nitrogen, mgm./10 ml.
Seitz filtered	2	3.93
Autoclaved	1	3.79
"	2	3.52
"	3	3.45

A loss of tryptophan was demonstrated by a lowered growth response. Typical curves obtained with *L. arabinosus* (standard microbiological procedures) are presented in Fig. 6*A*. Proof that the lowered growth response

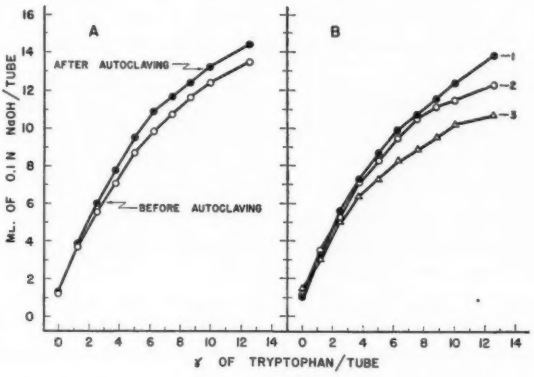

FIG. 6.

A. Effect of autoclaving on growth response to standard tryptophan levels.

B. Effect of dextrose on growth response to standard tryptophan levels.

1. 2% dextrose in medium, 0% in tryptophan standard.
2. 2% dextrose in medium, 10% in tryptophan standard.
3. 4% dextrose in medium, 10% in tryptophan standard.

was caused by destruction of tryptophan was obtained in a second experiment by adding, after autoclaving, amounts of tryptophan equivalent to the calculated amount destroyed. The resultant curve closely approximated that obtained when all of the tryptophan was added after autoclaving.

The effect of dextrose on the extent of the loss of tryptophan was tested by adding dextrose together with the tryptophan standard so that the amount of dextrose per tube increased progressively with increasing tryptophan. The results, presented graphically in Fig. 6*B*, show that the addition of dextrose along with the standard tryptophan significantly altered the growth response curve. This effect was more marked when the medium itself had a high dextrose content. It is thus apparent that a serious error may be introduced into microbiological assays for tryptophan if the sample to be assayed contains dextrose. or presumably any other aldehyde, and that the error is greater in media of high initial dextrose levels.

Discussion and Conclusions

Orla-Jensen (6) appears to have been first to recognize the stimulatory effect of autoclaved media on *Lactobacilli*. As a possible explanation he suggested the formation of a complex growth factor. Smiley, Niven, and Sherman (9) observed this phenomenon with *S. salivarius*, and showed that

caramelized dextrose, pyruvic acid, or acetaldehyde, had a similar effect. Niven and Sherman (5) failed to observe any difference in the growth stimulating properties of autoclaved and unautoclaved media when studying *S. faecalis* or *S. zymogenes*, but Rabinowitz and Snell (8) observed poor growth of *S. faecalis* in unautoclaved medium, and found that the addition of a reducing agent, such as ascorbic acid or sodium thioglycollate, overcame the deficiency of the medium.

The results of the present work are essentially in agreement with those quoted. The diverse results previously obtained with *S. faecalis* are, at least in part, explained by the fact that improved growth in autoclaved media was apparent only when a light inoculum was used. In agreement with the results of Rabinowitz and Snell (8) ascorbic acid has been found to stimulate initial growth of the *Lactobacilli*, but it appears probable that this effect was not entirely due to its reducing properties. Maillard products have mild reducing properties, but when formed externally and added to the medium, they failed to stimulate the initial growth. Furthermore, Seitz filtered medium and medium for which the dextrose had been autoclaved separately appeared to possess the same *Eh*, but the former usually supported better initial growth than the latter. Probably some additional factor is involved.

Under the conditions used in our studies, the apparent inhibitions due to moderate concentrations of Maillard product do not appear to have been true inhibitions, but were the result of loss of available nutrient concomitant to the formation of Maillard product. This finding is in agreement with that of Rabinowitz and Snell (8), who showed that, under the conditions used by them, destruction of cystine and cysteine was responsible for the lowered growth. In the present work, destruction of both dextrose and tryptophan was responsible for lowered growth, and probably any essential amino acid, if initially present in limiting quantity, would behave similarly. Thus, there appears to be little direct evidence of toxic effects traceable to Maillard products, especially as regards lactic acid bacteria.

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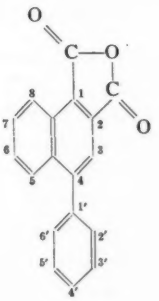
THE ULTRAVIOLET ABSORPTION SPECTRA OF SOME CARBOXY DERIVATIVES OF NAPHTHALENE[1]

BY Y. HIRSHBERG AND R. NORMAN JONES

Abstract

The ultraviolet absorption spectra of a variety of naphthalene compounds containing phenyl and carboxy substituents are described. The majority of the compounds contain either the naphthalene-1,2-dicarboxylic acid anhydride or the naphthalene-2,3-dicarboxylic acid anhydride ring systems. It is shown that in ethanolic solution the spectra of these anhydrides change over a period of a few hours. The spectra of the anhydrides in *n*-heptane or dioxane solution do not change on standing. The effects of the various substituents are discussed in terms of steric inhibition of resonance and of antagonistic and reinforcing actions of the substituents, dependent on the position of substitution. The significance of these data are considered in relation to the general problem of the interpretation of the ultraviolet absorption spectra of complex molecules.

A systematic investigation of the reaction between 1,1-diarylethylenes and maleic anhydride, carried out at the Daniel Sieff Research Institute by Bergmann, Szmuszkovicz, and Fawaz (2, 3, 21), has made available a series of derivatives of 4-phenylnaphthalene-1,2-dicarboxylic acid anhydride (I) in which the substituents are alkyl, aryl, halogen, and methoxy groups.* Certain of these derivatives exhibited very intense fluorescence, and this observation prompted an investigation of their fluorescence and ultraviolet absorption spectra. These studies were extended subsequently to include simpler naphthalene compounds containing phenyl and carboxyl groups, and to related compounds in the naphthalene-2,3-dicarboxylic acid anhydride



I

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* *Different systems of ring numbering are employed for these compounds in the publications cited in references (2) and (3). The system used in this paper corresponds with that adopted in the more recent publication (2) of Bergmann and Szmuszkovicz.*

series. The ultraviolet absorption spectra are described below, and the fluorescence spectra will be dealt with in a subsequent publication.

I. Effects of Conjugated Substituents on the Spectrum of Naphthalene

The ultraviolet absorption spectrum of naphthalene consists of three parts which are designated A, B, and C in Fig. 1. The result of introducing a

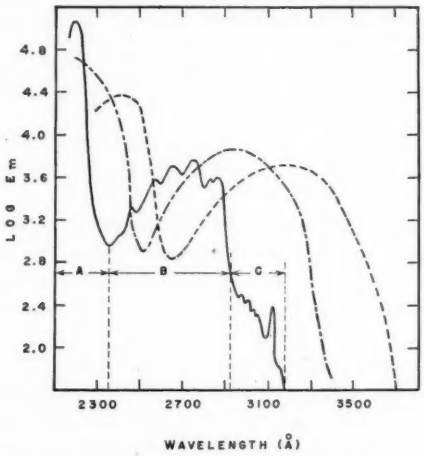


Fig. 1. *1-a* ——— *Naphthalene (ethanol)*
1-b – – – – *1-Naphthylamine (ethanol)*
1-c –·–·–·– *1-Naphthoic acid (ethanol)*

conjugatable substituent at position 1 is shown in the spectra of 1-naphthylamine and 1-naphthoic acid in Curves 1-*b* and 1-*c*. The 1-substituent produces a large bathochromic displacement and broadening of band A. The B and C groups of bands become fused into a single band group, probably as a result of the broadening and bathochromic displacement of the B band group and its superposition on the weaker C band group.

The introduction of a conjugated substituent at position 2 produces changes of a different character (Curves 2-*a*, 2-*b*). The displacement of the A band is similar to that produced by the 1-substituent, but the B band group is hardly influenced by the introduction of the 2-substituent. The C band group is displaced bathochromically and greatly intensified. The indifference of the B band group to increased conjugation at position 2 parallels similar effects seen in other series of polynuclear aromatic hydrocarbons, such as the 4-substituted pyrenes, which have been described previously (9). Specific effects of conjugatable substituents on localized regions of the absorption spectrum have been discussed at length in connection with the spectrum of anthracene (10), and an attempt has been made to relate these to the direction of polarization of the associated processes of electronic excitation. In line with these

arguments, it may be suggested that the B band group in naphthalene is related to an excitation directed along the *bb'*-axis of the molecule (II), and that its displacement in the 1-substituted derivatives results primarily from the

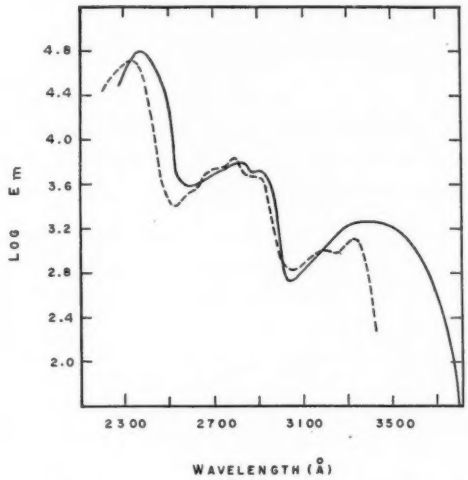
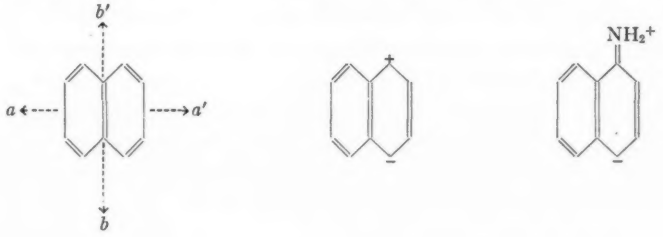


FIG. 2. *2-a* ——— *2-Naphthylamine (ethanol)*
2-b - - - - *2-Naphthoic acid (ethanol)*

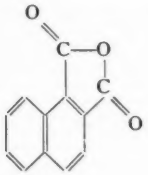
lowered energy of IV and related quinonoid structures in comparison with the corresponding structures (III) in the unsubstituted hydrocarbon.



II III IV

Further insight into the effects of conjugation on the naphthalene spectrum is provided by naphthalene-1,2-dicarboxylic acid anhydride (V). In this compound conjugation at both positions 1 and 2 is operative, and the presence of the two carbonyl groups in the pentacyclic anhydride ring assures the whole conjugated system is planar so that the minimum S-effect and maximum C-effect is manifested.* The spectrum in *n*-heptane solution

* *The symbols C (conjugation), S (steric), Fs (fine structure) and B (bathochromic) employed here to describe various categories of spectral shifts were introduced in an earlier paper (9) and described fully therein.*



V

(Curve 3-*a*) shows a further bathochromic displacement of band A. The group of absorption bands appearing between 3100 and 3600 Å can possibly be divided into B and C groups as indicated.

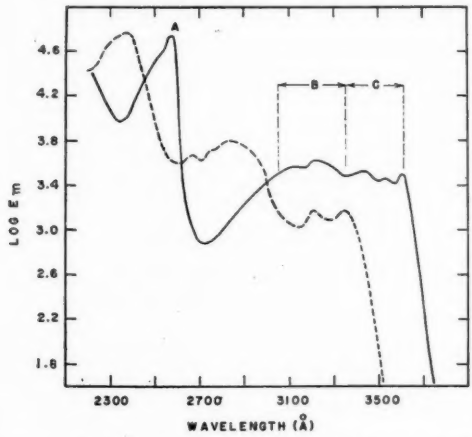


FIG. 3. 3-*a* ——— *Naphthalene-1,2-dicarboxylic acid anhydride* (*n-heptane*) (*V*)
3-*b* – – – – *Ditto* (*ethanol*)

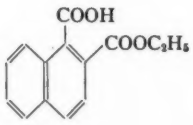
If this curve is compared with the spectrum of 1-naphthoic acid (1-*c*) an increase in the resolution of the fine structure at the longer wave lengths is the most prominent difference. This is in accord with previous observations (9) that such Fs effects are enhanced by cyclization.

The Reaction of Acid Anhydrides with Ethanol

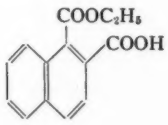
When naphthalene-1,2-dicarboxylic acid anhydride is dissolved in ethanol, the spectrum undergoes a *slow* change, and, after a few hours a new stable spectrum is established (3-*b*). This phenomenon occurred invariably in all the derivatives of both the 1,2- and the 2,3-anhydrides examined.

Siegel and Moran (20) have reported that acid anhydrides react *instantaneously* with primary alcohols to form hemiesters. According to this hypothesis, the products formed on solution of naphthalene-1,2-dicarboxylic acid anhydride in ethanol would possess a structure VI*a* and VI*b*. Recently the reaction proposed by Siegel and Moran has been subject to criticism. Anderson

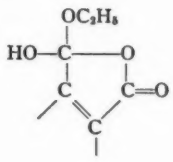
and Kenyon (1) could only isolate unchanged starting material after treating phthalic anhydride with ethanol for one hour at room temperature, while Lavine and Herkness (11) have questioned the interpretation of the titration data presented by Siegel and Moran in support of their hypothesis.



VI*a*



VI*b*



VII

The changes in the ultraviolet absorption spectra observed in our experiments clearly demonstrate that under the conditions employed a chemical reaction does occur between the anhydride and ethanol. It involves a modification of the conjugated system, and a partial or complete esterification would appear to be the most logical reaction. An alternative might be addition of ethanol at one of the carbonyl groups to yield a product analogous to a hemiacetal (VII). Such products would be readily decomposed and this might account for the failure of Anderson and Kenyon to isolate any reaction product from phthalic anhydride and ethanol.

Wolf and Herold (26) have observed that aliphatic aldehydes react readily in dilute ethanol solution to form hemiacetals with complete disappearance of the carbonyl absorption band.

Further studies of the nature of these reaction products are being undertaken at Rehovoth; in the subsequent discussion in this paper the substances produced will be referred to merely as "ethanolysis products".

The spectrum of naphthalene-1,2-dicarboxylic acid anhydride is distinctly of the 1-substituted type, from which it may be argued that structures of type VIII in which the negative charge rests on the 1-carbonyl oxygen atom exert the dominant effect in determining the energy levels. On ethanolysis the spectrum changes to a 2-substituted type. In the ethanolysis product, therefore, the relative importance to the resonance of VIII and IX type structures is inverted.*

The spectrum of naphthalene-2,3-dicarboxylic acid anhydride (X) in *n*-heptane solution is shown in Curve 4-*a*. The structure of this compound provides for a maximal excitation of the 2-conjugated type of naphthalene substitution. In this spectrum the B band group is least influenced by the substituents, while the greatest effect is on the C band group, which is greatly

** If the ethanolysis product is a hemiester, the carboxyl or carboethoxy group at position 1 will be highly hindered by the large group at 2. This will force it out of the plane of the ring system and the hypsochromic displacement of the absorption must be attributed to the diminished conjugation between the 1-carbonyl group and the ring. The 2-carbonyl group, being hindered on one side only, will be less affected. If the ethanolysis results in addition of ethanol at one of the carbonyl groups (VII), it is the 1-carbonyl that would appear to be involved.*

intensified and displaced bathochromically. These changes are similar in type, but greater in magnitude, than those that result from the introduction of the 2-carboxylic acid group.

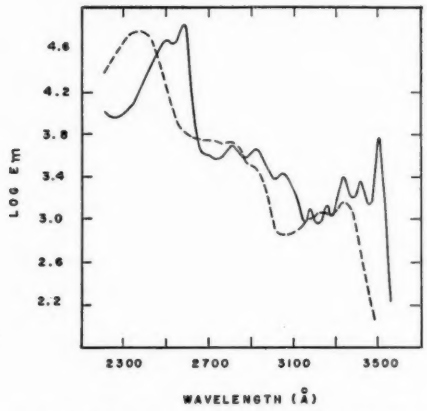
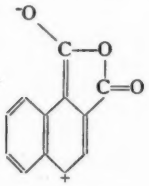
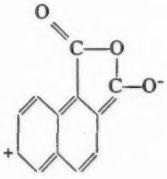


FIG. 4. *4-a* ——— *Naphthalene-2,3-dicarboxylic acid anhydride (n-heptane) (X)*
4-b - - - - *Ditto (ethanol)*

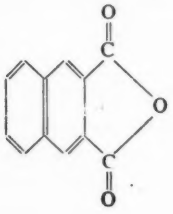


VIII



IX

In solution in ethanol the spectrum of naphthalene-2,3-dicarboxylic acid anhydride changes, and the stable spectrum that is finally established (Curve 4-*b*) closely resembles that of 2-naphthoic acid (Curve 2-*b*).



X

II. Phenyl Derivatives of Naphthalene

The curves of 1-phenylnaphthalene (5-*a*) and 1-naphthoic acid (5-*b*) are similar, and may be contrasted with the spectrum of 1-naphthalacetone (XI)

(5-*c*) reproduced from the data of Wilds *et al.* (25). Friedel, Orchin, and Reggel, who have reported on the spectra of several arylnaphthalenes (7), consider that in the 1-phenyl derivative the phenyl group is subject to strong

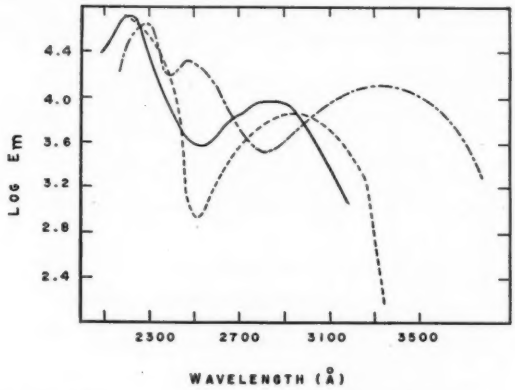
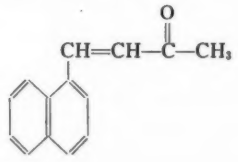


FIG. 5. 5-*a* ——— *1-Phenylnaphthalene (ethanol)*
5-*b* - - - - *1-Naphthoic acid (ethanol)*
5-*c* -·-·-·- *1-Naphthalacetone (ethanol)* [*XI*]

steric hindrance, and that coupling between the benzene and naphthalene chromophores is sensibly diminished on this account. Comparison with the



XI

spectrum of naphthalene and the other 1-substituted derivatives shown in Fig. 1 suggests that, although there is a significant S-effect in 1-phenylnaphthalene, it is by no means comparable with the strong steric effects noted in such compounds as 9,10-diphenylanthracene (10).

In Curves 6-*a*, 6-*b*, and 6-*c* the spectra of 2-phenylnaphthalene, 2-naphthoic acid, and 2-naphthalacetone are shown. The effects of steric factors on the spectrum of 2-phenylnaphthalene has been discussed previously (7, 8); in all these compounds unhindered planar structures may be postulated. Whereas the 2-carboxy and 2-phenyl groups leave the B band group of naphthalene relatively undisturbed, in the 2-naphthalacetone more complex changes occur.*

* *In 2-phenylnaphthalene, structure analogous to the C absorption bands of 2-naphthylamine and 2-naphthoic acid is seemingly lacking. However, the measurements of the spectrum of 2-phenylnaphthalene have not been extended to log E_m values below 2.8.*

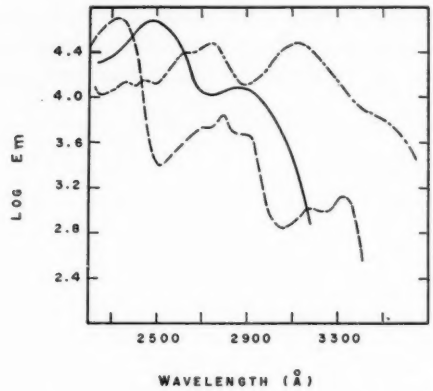


FIG. 6. *6-a* ——— *2-Phenylnaphthalene (ethanol)*
6-b – – – – *2-Naphthoic acid (ethanol)*
6-c –·–·–·– *2-Naphthalacetone (ethanol)*

III. 4-PHENYLNAPHTHALENE-1,2-DICARBOXYLIC ACID ANHYDRIDE

The spectrum of 4-phenylnaphthalene-1,2-dicarboxylic acid anhydride is shown in Curve 7-*a*. Comparison with 7-*b* indicates that the introduction of

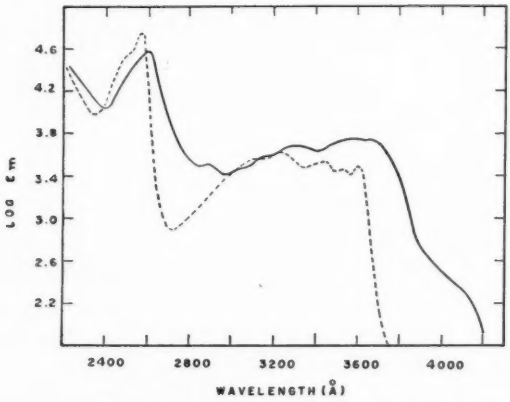


FIG. 7. *7-a* ——— *4-Phenylnaphthalene-1,2-dicarboxylic acid anhydride (n-heptane) [I]*
7-b – – – – *Naphthalene-1,2-dicarboxylic acid anhydride (n-heptane) [V]*

the 4-phenyl substituent causes significant changes. In the B and C band groups, the maxima are displaced bathochromically by 100 Å; the A band, however, is hardly affected. At long wave lengths the spectrum slopes off gradually and there is an indication of a new low intensity band extending into the visible.

In ethanolic solution the anhydride undergoes alcoholysis. The spectrum of the reaction product (8-*a*) closely resembles that of the monoester of V (8-*b*) save only for the band at 3900 Å. The similarity of 8-*a* and 8-*b* above 3500 Å

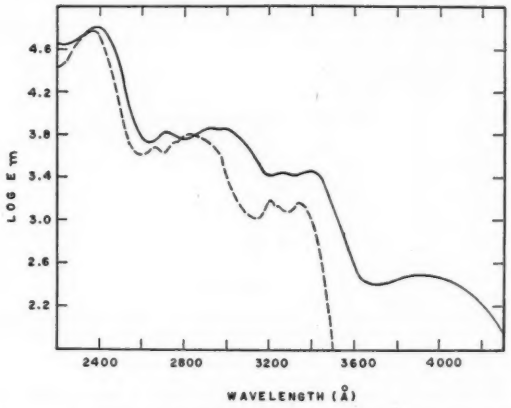


FIG. 8. *8-a* ——— *4-Phenylnaphthalene-1,2-dicarboxylic acid anhydride (ethanol)*
8-b – – – – *Naphthalene-1,2-dicarboxylic acid anhydride (ethanol)*

is in accord with the observations made above that the conjugation in the molecule is diminished on reaction with ethanol.

The low intensity band at 3900 Å is difficult to account for. It was at first considered that it might arise from a trace of a strongly absorbing impurity, but this was discounted by the fact that similar bands are seen also in the spectra of a large number of derivatives of 4-phenylnaphthalene-1,2-dicarboxylic acid anhydride prepared by independent syntheses. It is not influenced by the opening of the anhydride ring and its greater prominence in the spectra of the ethanolysis products results from the hypsochromic displacement of the remainder of the spectrum on ethanolysis.

IV. Alkyl, Halogen, and Methoxyl Derivatives of 4-Phenylnaphthalene-1,2-dicarboxylic Acid Anhydride

The introduction of a methyl group at the 4′ position into 4-phenylnaphthalene-1,2-dicarboxylic acid anhydride does not influence the general shape of the absorption curve; there is a small bathochromic displacement of the A and C groups of bands while the B band group is scarcely changed (Curve 9-*a*). This is true both of the spectra of the anhydride and of its ethanolysis product. The positions of the absorption maxima in the 4′-methyl, 4′-ethyl, 4′-isopropyl, 4′-tert. butyl derivatives are identical within the experimental limits of measurements (Table I). In this series of compounds there is no

indication of a hypsochromic shift in passing from the methyl to higher branched chain alkyl derivatives such as Matsen, Robertson, and Chuoke observed in a similar series of alkyl benzenes (13).

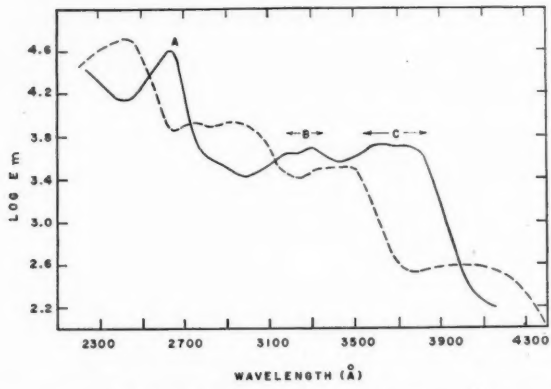
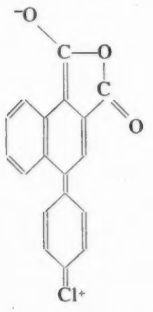
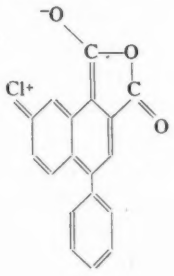


FIG. 9. *9-a* ——— *4′-Methyl-4-phenylnaphthalene-1,2-dicarboxylic acid anhydride (n-heptane)*
9-b – – – – *Ditto (ethanol)*

Several halogen derivatives of 4-phenylnaphthalene-1,2-dicarboxylic anhydride have also been examined. These are listed in Table I, and contain fluorine, chlorine, and bromine substituents at positions 4′ and 7. They also yield spectra that are almost identical with those of the 4′-methyl derivative. Although quinonoid structures like XII and XIII may be written for these compounds, their contributions to the resonance stabilization of the excited states must be comparable with that of the analogous structures in the corresponding alkyl derivatives, where the positive charge is on a carbon atom of the ring. This is in agreement with observations on the spectra of halogen derivatives of other polynuclear aromatic hydrocarbons such as 2-chlorophenanthrene (9).



XII



XIII

TABLE I

WAVE LENGTHS AND INTENSITIES OF THE ABSORPTION MAXIMA

Compound	Solvent[g]	Source[a]	Position of absorption maxima[b]	
			Wave length, Å	Intensity log E molar
Naphthalene	Ethanol	—	[c]	
1-Naphthylamine	Ethanol	—	2400	4.36
			3220	3.71
2-Naphthylamine	Ethanol	—	[d]	
1-Naphthoic Acid	Ethanol	i		
2-Naphthoic Acid	Ethanol	i		
Naphthalene-1,2-dicarboxylic acid anhydride	*n*-Heptane	ii	(2480)	4.54
			2560	4.74
			3080	3.59
			(3220)	3.56
			3440	3.54
			3540	3.45
			3600	3.50
	Ethanol		2680	3.67
			(2750)	3.71
			2820	3.83
			3200	3.19
			3340	3.18
Naphthalene-2,3-dicarboxylic acid anhydride	*n*-Heptane	iii	2500	4.58
			2570	4.78
			2810	3.69
			2930	3.67
			3040	3.44
			3180	3.10
			3260	3.15
			3340	3.45
			3420	3.36
			3500	3.81
	Ethanol		2360	4.77
			2710	3.72
			2800	3.74
			(2920)	3.48
			(3180)	3.02
			3220	3.06
			3340	3.12
1-Phenylnaphthalene	Ethanol	—		[c]

[a] i. *Eastman Kodak Company.*
ii. *Compound synthesized by F. Bergmann and J. Szmuszkovicz.*
iii. *Compound synthesized by E. Bergmann.*

[b] *The more pronounced inflections are indicated by figures in parentheses.*

[c] *See Friedel, Orchin, and Reggel (Ref. 7).*

[d] *See Jones (Ref. 10).*

[g] *The spectra in n-heptane or dioxane solution were always quite similar. Major differences occur on solution in ethanol (see page 440).*

TABLE I—*Continued*

WAVE LENGTHS AND INTENSITIES OF THE ABSORPTION MAXIMA—*Continued*

Compound	Solvent[g]	Source[a]	Position of absorption maxima[b]	
			Wave length, Å	Intensity log *E* molar
1-Naphthalacetone	95% Ethanol	—		[e]
2-Phenylnaphthalene	Ethanol	—		[f]
2-Naphthalacetone	Ethanol	—		[e]
4-Phenylnaphthalene-1,2-dicarboxylic acid anhydride	*n*-Heptane	ii	2600	4.61
			2880	3.52
			(3160)	3.58
			3300	3.70
			3570	3.75
			3660	3.73
	Ethanol		2380	4.77
			2720	3.81
			2910	3.86
			2990	3.86
			(3250)	3.45
			3400	3.48
			3880	2.50
4′-Methyl-4-phenylnaphthalene-1,2-dicarboxylic acid anhydride	*n*-Heptane	ii	2645	4.64
			3200	3.64
			3300	3.70
			3620	3.72
			3750	3.70
	Ethanol		2450	4.72
			2750	3.92
			2910	3.94
			(3350)	3.50
			3450	3.50
			4000	2.61
4′-Ethyl-4-phenylnaphthalene-1,2-dicarboxylic acid anhydride	*n*-Heptane	ii	2220	4.46
			2650	4.65
			3200	3.61
			3300	3.70
			3620	3.70
			3750	3.70
	Ethanol		2420	4.74
			2750	3.98
			3450	3.48
			4000	2.80

[a] ii. *Compound synthesized by F. Bergmann and J. Szmuszkovicz.*

[b] *The more pronounced inflections are indicated by figures in parentheses.*

[e] *See Wilds et al. (Ref. 25).*

[f] *See Jones (Ref. 8).*

[g] *The spectra in n-heptane or dioxane solution were always quite similar. Major differences occur on solution in ethanol (see page 440).*

TABLE I—*Continued*

WAVE LENGTHS AND INTENSITIES OF THE ABSORPTION MAXIMA—*Continued*

Compound	Solvent[c]	Source[a]	Position of absorption maxima[b]	
			Wave length, Å	Intensity log *E* molar
4′-Isopropyl-4-phenylnaphthalene-1,2-dicarboxylic acid anhydride	*n*-Heptane	ii	2220	4.44
			2655	4.61
			3200	3.71
			3320	3.64
			3620	3.65
			3745	3.65
	Ethanol		2460	4.79
			2760	4.05
			3440	3.56
4′-Tert. butyl-4-phenylnaphthalene-1,2-dicarboxylic acid anhydride	*n*-Heptane	ii	2660	4.75
			3210	3.69
			3325	3.77
			3620	3.81
			3745	3.74
	Ethanol		2460	4.84
			2780	3.91
			2940	3.99
			3320	3.59
			3450	3.69
			4000	2.34
4′,7-Dimethyl-4-phenylnaphthalene-1,2-dicarboxylic acid anhydride	*n*-Heptane	ii	2645	4.65
			3000	3.58
			(3220)	3.71
			3340	3.80
			3660	3.81
			3745	3.81
	Ethanol		2440	4.75
			2760	3.80
			2960	3.92
			(3360)	3.56
			3450	3.60
			4000	2.38
4′-Fluoro-4-phenylnaphthalene-1,2-dicarboxylic acid anhydride	*n*-Heptane	ii	2600	4.65
			(2890)	3.46
			(3190)	3.70
			3300	3.76
			3560	3.80
			3640	3.79
	Ethanol		2420	4.75
			(2750)	3.72
			2940	3.87
			2990	3.88
			3280	3.48
			3400	3.50

[a] ii. *Compound synthesized by F. Bergmann and J. Szmuszkovicz.*

[b] *The more pronounced inflections are indicated by figures in parentheses.*

[c] *The spectra in n-heptane or dioxane solution were always quite similar. Major differences occur on solution in ethanol* (see page 440).

TABLE I—*Continued*

WAVE LENGTHS AND INTENSITIES OF THE ABSORPTION MAXIMA—*Continued*

Compound	Solvent[c]	Source[a]	Position of absorption maxima[b]	
			Wave length, Å	Intensity log *E* molar
4′-Chloro-4-phenylnaphthalene-1,2-dicarboxylic acid anhydride	1,4-Dioxane	ii	2620	4.66
			(2900)	3.67
			3340	3.78
			3610	3.84
			3640	3.83
	Ethanol		2420	4.74
			2730	3.86
			3000	3.97
			3400	3.58
			(4000)	2.40
4′-Bromo-4-phenylnaphthalene-1,2-dicarboxylic acid anhydride	*n*-Heptane	ii	2660	4.64
			(3170)	3.76
			3300	3.82
			3580	3.73
			3720	3.73
4′-Fluoro-7-methyl-4-phenylnaphthalene-1,2-dicarboxylic acid anhydride	*n*-Heptane	ii	2645	4.64
			(3170)	3.68
			3310	3.68
			3630	3.69
			3750	3.69
	Ethanol		2450	4.62
			2750	3.89
			2900	3.89
			(3300)	3.46
			3450	3.46
			4000	2.60
4′,7-Dichloro-4-phenylnaphthalene-1,2-dicarboxylic acid anhydride	*n*-Heptane	ii	2635	4.62
			(3170)	3.72
			3305	3.79
			3580	3.71
			3720	3.73
	Ethanol		2400	4.71
			2950	3.94
			3400	3.50
			4000	2.38
7-Methoxy-4-phenylnaphthalene-1,2-dicarboxylic acid anhydride	*n*-Heptane	ii	2260	4.78
			2695	4.60
			(3180)	3.70
			3300	3.75
			3795	3.69
			3980	3.73
	Ethanol		2440	4.62
			2780	4.01
			(2900)	3.92
			(3440)	3.40
			3580	3.44
			4180	2.78

[a] ii. *Compound synthesized by F. Bergmann and J. Szmuszkovicz.*

[b] *The more pronounced inflections are indicated by figures in parentheses.*

[c] *The spectra in n-heptane or dioxane solution were always quite similar. Major differences occur on solution in ethanol* (*see page 440*).

TABLE I—*Continued*

WAVE LENGTHS AND INTENSITIES OF THE ABSORPTION MAXIMA—*Continued*

Compound	Solvent[c]	Source[a]	Position of absorption maxima[b]	
			Wave length, Å	Intensity log *E* molar
4′,7-Dimethoxy-4-phenylnaphthalene-1,2-dicarboxylic acid anhydride	1,4-Dioxane	ii	2720	4.48
			(3200)	3.64
			3440	3.83
			3880	3.83
			4000	3.84
	Ethanol		2430	4.67
			3000	3.80
			3370	3.53
			3600	3.65
			4260	2.52
6,7-Dimethoxy-4-phenylnaphthalene-1,2-dicarboxylic acid anhydride	*n*-Heptane	ii	2340	4.68
			2750	4.57
			3600	4.02
	Ethanol		2580	4.74
			3080	4.04
			3920	2.60
4,7-Diphenylnaphthalene-1-carboxylic acid	Ethanol	ii	2420	4.82
			(2800)	4.50
			3740	3.54
1,6-Diphenylnaphthalene	Ethanol	ii	(2350)	4.50
			2580	4.73
			(3000)	4.00
4,7-Diphenylnaphthalene-1,2-dicarboxylic acid anhydride	1,4-Dioxane	ii	2820	4.52
			3440	3.77
			3760	3.64
	Ethanol		2460	4.74
			2620	4.71
			3400	3.35
			(3550)	3.32
4,1′-Dinaphthyl-1,2-dicarboxylic acid anhydride	1,4-Dioxane	ii	2240	4.97
			2600	4.62
			(2730)	4.15
			(2820)	4.00
			(2920)	3.87
			(3160)	3.62
			3320	3.70
			3620	3.76
	Ethanol		2240	4.81
			2380	4.70
			2820	4.07
			(3250)	3.55
			3380	3.55

[a] ii *Compound synthesized by F. Bergmann and J. Szmuszkovicz.*

[b] *The more pronounced inflections are indicated by figures in parentheses.*

[c] *The spectra in n-heptane or dioxane solution were always quite similar. Major differences occur on solution in ethanol* (*see page 440*).

TABLE I—*Concluded*

WAVE LENGTHS AND INTENSITIES OF THE ABSORPTION MAXIMA—*Concluded*

Compound	Solvent[c]	Source[a]	Position of absorption maxima[b]	
			Wave length, Å	Intensity log *E* molar
1-Phenylnaphthalene-2,3-dicarboxylic acid anhydride	1,4-Dioxane	iii	2580	4.74
			3000	3.80
			3100	3.80
			3440	3.57
			3580	3.70
	Ethanol		2280	4.65
			2400	4.73
			(2700)	3.70
			2850	3.80
			3240	3.20
			3380	3.26

[a] iii. *Compound synthesized by E. Bergmann.*

[b] *The more pronounced inflections are indicated by figures in parentheses.*

[c] *The spectra in n-heptane or dioxane solution were always quite similar. Major differences occur on solution in ethanol* (*see page 440*).

The introduction of a methoxy group at position 7 significantly alters the spectrum (Curve 10-*a*). Both the A and C band groups are displaced bathochromically, although the B bands are hardly affected. The introduction

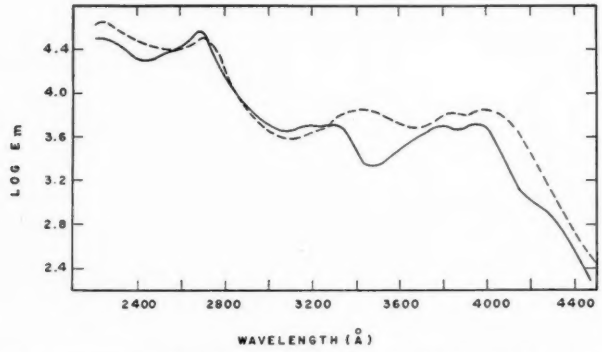


FIG. 10. *10-a* ——— *7-Methoxy-4-phenylnaphthalene-1,2-dicarboxylic acid anhydride (n-heptane)*

10-b – – – – *4′,7-Dimethoxy-4-phenylnaphthalene-1,2-dicarboxylic acid anhydride (dioxane) [XIV]*

of a second methoxyl group in the 4′,7-dimethoxy derivative has a slight additional influence on the A and C bands and also displaces the B bands (Curve 10-*b*). In both of these methoxy derivatives ethanolysis causes a reversion

to a spectrum like that of the ethanolysis product of the nonmethoxylated compound (Curves 11-*a*, 11-*b*, 11-*c*).

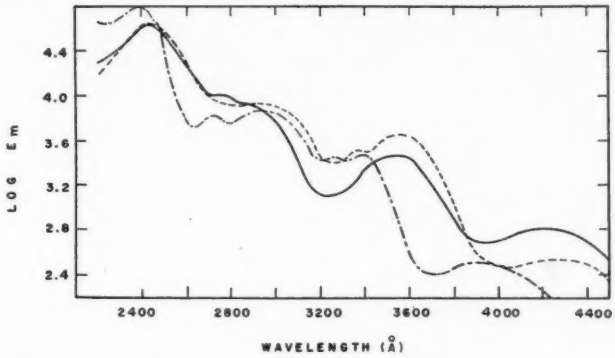


FIG. 11. *11-a* ——— *7-Methoxy-4-phenylnaphthalene-1,2-dicarboxylic acid anhydride (ethanol)*
11-b - - - - *4′,7-Dimethoxy-4-phenylnaphthalene-1,2-dicarboxylic acid anhydride (ethanol)*
11-c -·-·-·- *4-Phenylnaphthalene-1,2-dicarboxylic acid anhydride (ethanol)*

In 6,7-dimethoxynaphthalene-1,2-dicarboxylic acid anhydride (XIV), introduction of the 6-methoxy group causes the absorption bands to shift *hypsochromically* (12-*a*); the methoxyl groups at 6 and 7 seem to exercise opposing

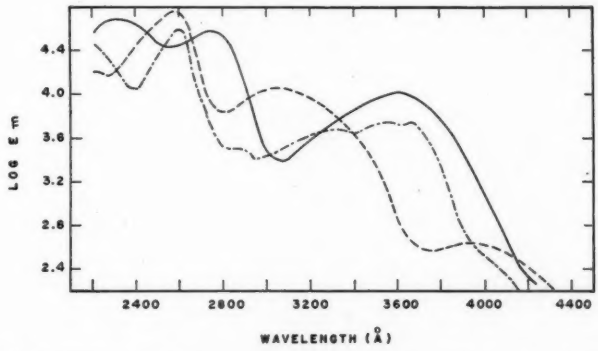
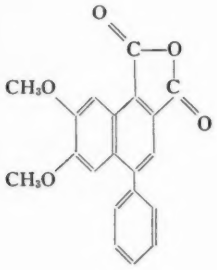


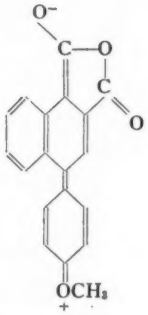
FIG. 12. *12-a* ——— *6,7-Dimethoxy-4-phenylnaphthalene-1,2-dicarboxylic acid anhydride (dioxane)*
12-b - - - - *Ditto. (ethanol)*
12-c -·-·-·- *4-Phenylnaphthalene-1,2-dicarboxylic acid anhydride (n-heptane)* [*1*]

influences. Methoxy groups at 4′ and 7 may exert their major effect through the participation in the resonance of structures typified by XV and XVI, in which the negative charge is on the carbonyl oxygen atom at position 1.

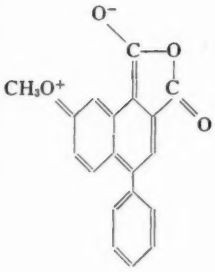
Such quinonoid structures cannot be written for 4′- and 7-methoxy derivatives in which the charge is transferred to the carbonyl oxygen atom at 2. In the case of the 6-methoxy compound the opposite is the case (XVII). In the 6,7-dimethoxy derivative there is, therefore, a competitive action between the stabilizing effects of the two methoxyl groups on the two carbonyl groups, and the abnormal effects on the spectrum may result from this crossed conjugation.



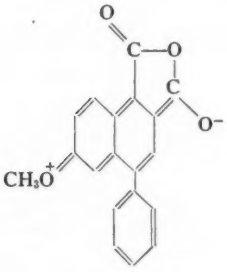
XIV



XV



XVI



XVII

V. Other Aryl Derivatives of Naphthalene-1,2-dicarboxylic anhydride

The spectra of 4,7-diphenylnaphthalene-1-carboxylic acid (XVIII) and the corresponding hydrocarbon (XIX) are compared in Curves 13-*a* and 13-*b*. The spectrum of the hydrocarbon resembles that of 2-phenylnaphthalene (13-*c*) but the introduction of the 1-carboxyl group produces a broad new band extending into the visible region of the spectrum. Conjugated substituents at 4 and 7 will exercise a reinforcing action on the 1-quinonoid excited states (cf. XV, XVI) and steric inhibition of resonance will be slight in the

absence of substituents at 2 and 6. These conditions are favorable for the stabilization of the excited states of the 1-carboxylic acid, and the long wave absorption band is understandable.

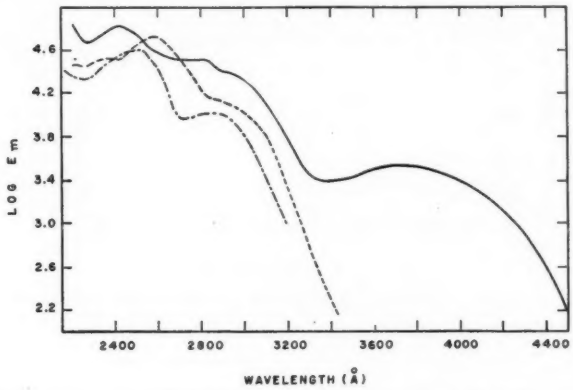
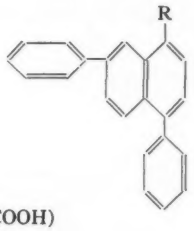
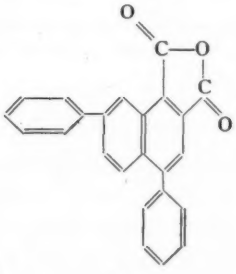


FIG. 13. *13-a* ——— *4,7-Diphenylnaphthalene-1-carboxylic acid (ethanol) [XVIII]*
13-b – – – – *1,6-Diphenylnaphthalene (ethanol) [XIX]*
13-c –·–·–·– *2-Phenylnaphthalene (ethanol)*

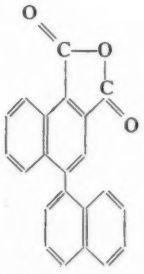


XVIII (R = −COOH)
XIX (R = −H)

If the 1-carboxylic acid group is replaced by the 1,2-dicarboxylic acid anhydride ring (XX) Curve 14-*a* is obtained, and on ethanolysis the spectrum shifts hypsochromically (14-*b*).



XX



XXI

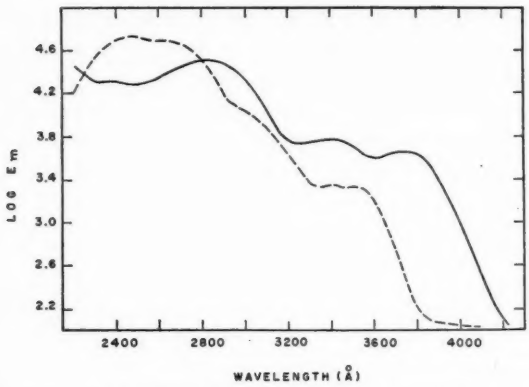


FIG. 14. *14-a* ——— *4,7-Diphenylnaphthalene-1,2-dicarboxylic acid anhydride (dioxane)* [*XX*]
14-b – – – – *Ditto. (ethanol)*

The spectrum of 4,1′-dinaphthyl-1,2-dicarboxylic acid anhydride (XXI) shown in Curve 15-*a* is quite similar to that of I (15-*b*), and the spectra of the corresponding ethanolysis products are also similar (16-*a*, 16-*b*).

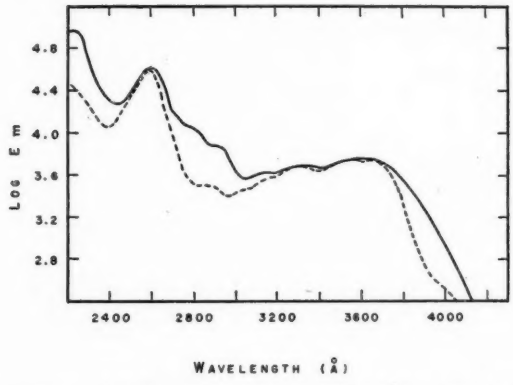


FIG. 15. *15-a* ——— *4,1′-Dinaphthyl-1,2-dicarboxylic acid anhydride (dioxane)* [*XXI*]
15-b – – – – *4-Phenylnaphthalene-1,2-dicarboxylic acid anhydride (n-heptane)* [*I*]

VI. Derivatives of Naphthalene-2,3-dicarboxylic Acid Anhydride

The spectrum of naphthalene-2,3-dicarboxylic acid anhydride (X) has been discussed in Section I. The introduction of the 1-phenyl group (XXII) greatly diminishes the fine structure (Curve 17-*a*) and displaces the B band group by 150 Å; the spectra of the ethanolysis products of both compounds are alike (18-*a*, 18-*b*).

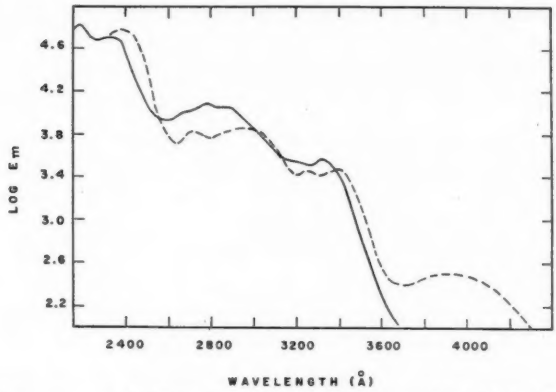


FIG. 16. *16-a* ——— *4,1'-Dinaphthyl-1,2-dicarboxylic acid anhydride (ethanol)*
16-b – – – – *4-Phenylnaphthalene-1,2-dicarboxylic acid anhydride (ethanol)*

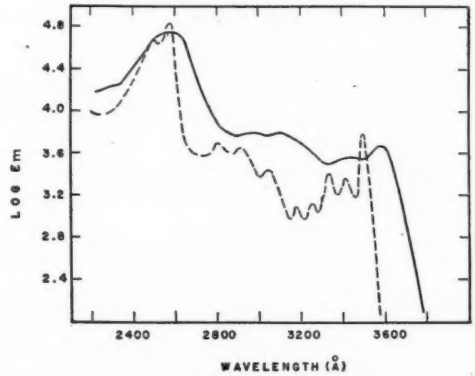


FIG. 17. *17-a* ——— *1-Phenylnaphthalene-2,3-dicarboxylic acid anhydride (dioxane) [XXII]*
17-b – – – – *Naphthalene-2,3-dicarboxylic acid anhydride (n-heptane) [X]*

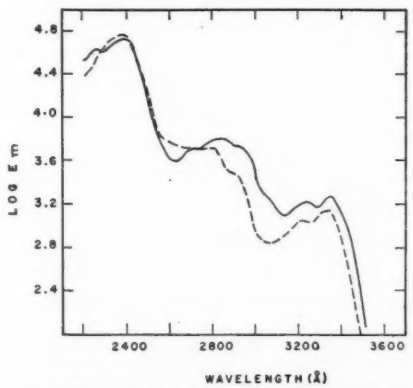
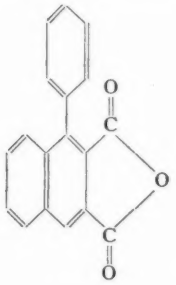


FIG. 18. *18-a* ——— *1-Phenylnaphthalene-2,3-dicarboxylic acid anhydride (ethanol)*
18-b – – – – *Naphthalene-2,3-dicarboxylic acid anhydride (ethanol)*

In this series of compounds it seems that provided the anhydride ring is present the phenyl substituent couples strongly with the naphthalene ring system, but this effect is depressed on reaction with ethanol.



XXII

Discussion

As yet, the quantum mechanical analysis of the electronic levels of organic compounds has not been developed sufficiently to provide a rigid interpretation of the ultraviolet absorption spectra of such complex molecules as are considered here. The recent work of Coulson (4), Mullikan (14, 15), Walsh (23), Pullman, Pullman, and Daudel (5, 6, 16, 17), and others, however, indicates that considerable progress is being made in this direction.

One of the primary objects of this paper, as of earlier papers from this laboratory (8, 9, 10), is to present spectrographic data on complex compounds, and to draw attention to certain empirical relationships between the spectra and the molecular structure which are of value in the elucidation of molecular structure and which must eventually be brought within the framework of the quantum mechanical treatment.

To this end, it is convenient to make use of quasi-classical concepts of the process of electronic excitation, in order to provide a basis for the orderly and rational presentation of the data and to emphasize certain correlations between spectra and molecular structure. It is not considered to be a matter of undue concern if on the basis of these simple concepts the interpretation lacks completeness or even leads to occasional inconsistencies.

For the purpose of description, the naphthalene spectrum has been divided into three groups of bands, designated A, B, and C, and there can be little doubt that, in naphthalene itself, these correspond to three separate states of electronic excitation.* In the simpler derivatives the same three groups of bands are clearly to be distinguished. In the more highly substituted compounds the description of the spectra in terms of A, B, and C regions is

* *The electronic states associated with the various regions of the naphthalene spectrum have been described also by West (24).*

retained as a matter of convenience, but it is by no means certain that these separate parts of the spectrum bear much more than a formal relation to the three well defined regions of the naphthalene spectrum. This comment applies particularly to the C region.

The postulate of Scheibe (18, 19) and of Lewis and Calvin (12) that the directions of electronic excitation in the plane of the molecule can be resolved into orthogonal vectors giving rise to x-bands and y-bands has been used previously in interpreting the effects of substituents on the spectrum of anthracene (10). In the naphthalene compounds discussed here, distinctions between the effects of the introduction of conjugated substituents at positions 1 and 2 can be explained in terms of the orientation of the exciting polarization provided the conjugated substituents are small, but the picture gets confused when the conjugation becomes more extensive as in the 1- and 2-naphthalacetones.

In the unsubstituted naphthalene molecule, considerations of symmetry would require the directions of such oriented polarization vectors to correspond closely with the symmetry axes in the plane of the molecule. Since the direction of the dipole in the 1-amino group lies along the bb'-axis (II), its primary effect will be restricted to the excitation of the B band. In more complex substituents, where the symmetry in the plane of the molecule is diminished by the extension of the conjugated chain, it is not possible to postulate the directions of x-band and y-band excitation from considerations of molecular geometry alone. In the general case the dipole moment of the substituent will have components in the directions of both the aa'- and bb'-axes, and any change in the conjugated substituents is liable to alter both the magnitude and the direction of the exciting polarization vectors. Therefore, it is only for small substituents, in molecules possessing a high degree of symmetry, that this analysis of substituent effects in terms of x-bands and y-bands can be applied effectively.

Another guiding principle that has aided in the rational interpretation of these substitution effects is the steric inhibition of resonance. In several instances (e.g., 9,10-diphenylanthracene) it has been clearly established that this effect may be sufficiently strong to inhibit completely any interaction between the aromatic ring and a potentially conjugatable substituent.

In the naphthalene series, it would appear that steric inhibition of resonance is more or less complete for the 1-substituted carboxy group, provided a second carboxy group, or other large substituent, is present at the vicinal 2-position. In the absence of a large substituent at 2, the naphthalene-1-carboxyl system is hindered only slightly.

A further factor influencing these substitution effects is the mutual reinforcing or antagonistic actions of two or more substituents in the same molecule. Such an antagonistic action has been postulated to account for the

differences in the spectra of 7-methoxy and 6,7-dimethoxy-4-phenylnaphthalene-1,2-dicarboxylic acid anhydride. This type of effect warrants further investigation in simpler compounds amenable to an analysis of the electron density distribution by the method of Pullman, Pullman, and Daudel.

In addition to these specific effects, the introduction of a substituent tends to shift the whole spectrum to longer wave lengths (B effect). This is seen most clearly following the introduction of alkyl substituents at positions where they do not increase steric inhibition of resonance. There is both experimental (10) and theoretical evidence (22) to indicate that this B effect acts primarily by raising the ground state of the molecule and it tends to produce a bodily shift of the whole spectrum. In the majority of the naphthalene compounds discussed here this effect is masked by conjugation effects, but it is seen in the alkyl and halogen derivatives of 4-phenylnaphthalene-1,2-dicarboxylic acid anhydride (Curve 9-*a*).

Experimental

The majority of the compounds discussed in this paper were prepared at the Daniel Sieff Research Institute and the methods of synthesis and proofs of structure have been described elsewhere (2, 3, 21). The spectra were determined on a Beckman model DU spectrophotometer using a constant band width of 10 Å.

Acknowledgment

We wish to express our indebtedness to Drs. E. Bergmann, F. Bergmann, and J. Szmuszkovicz who kindly placed at our disposal the majority of the compounds on which these studies are based. We also wish to thank Mr. A. Cahn, Miss Kathleen McLean, Miss M. Russell, and Miss L. Groth for technical assistance in the measurement of the spectra.

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NITROLYSIS OF HEXAMETHYLENETETRAMINE

II. NITROLYSIS OF 1,5-*ENDO*METHYLENE-3,7-DINITRO-1,3,5,7-TETRAZACYCLOÖCTANE (DPT)[1]

By A. F. McKay, H. H. Richmond[2], and George F Wright

Abstract

When 1,5-*endo*methylene-3,7-dinitro-1,3,5,7-tetrazacycloöctane (DPT) is nitrolyzed with nitric acid – ammonium nitrate mixture the products are cyclic trimeric and tetrameric methylenenitramines (RDX and HMX). When the ammonium nitrate in this nitrolysis mixture is replaced by anhydrides such as nitrogen pentoxide or acetic anhydride then terminally esterified linear polymethylenenitramines such as 1,9-dinitroxy-2,4,6,8-tetranitro-2,4,6,8-tetrazanonane and the 1,9-diacetoxy analogue respectively are obtained. Replacement of this nitric acid – anhydride mixture by acetyl nitrate does not produce the same type of nitrolysis. It is therefore concluded that nitric acid and an anhydride act independently, the former as a nitrolyzing agent and the latter as an esterifying agent. Alternatively the presence of ammonium nitrate serves to promote esterification and/or promote demethylolation.

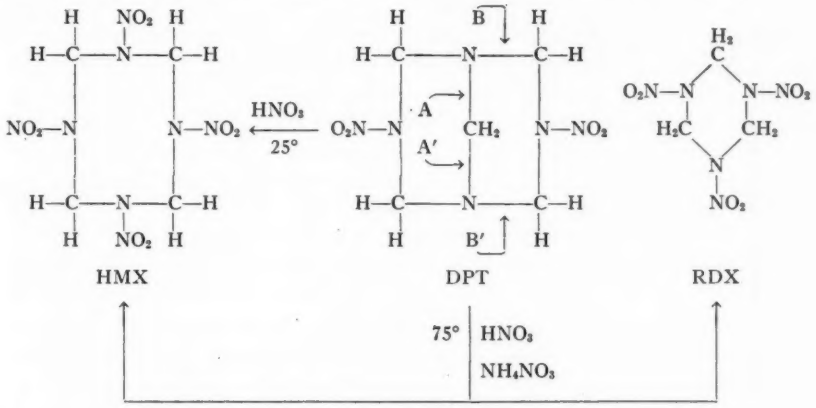
The preparation of 1,5-*endo*methylene-3,7-dinitro-1,3,5,7-tetrazacycloöctane (DPT) in anhydrous medium has been described (3) as a double scission at the amine nitrate linkages of hexamethylenetetramine (hexamine) dinitrate. The reaction conditions are mild and the amount of nitrolyzing acid is limited (to 2 equivalents). The resulting DPT is by no means stable to nitric acid. This report describes the subsequent scissions that it undergoes.

The dual nitrolysis in 99.6% nitric acid at 25° C. at the *endo*methylene linkage A,A′ has been described (3). No isolable product could be discovered when DPT was heated to 70°–75° C. with 99.6% nitric acid, but the milder reagent ammonium nitrate – nitric acid (1 : 1.78 molar) produced a mixture of Cyclonite (RDX) and 1,3,5,7-tetranitro-1,3,5,7-tetrazacycloöctane (HMX) According to thermal analysis of this mixture, the yields approximated 52% RDX and 17% HMX. When the ammonium nitrate – nitric acid ratio was 1 : 1, the RDX yield was slightly higher (57%) while the HMX yield became lower. The mode by which the two products are evolved is shown in the following formulation as scission at A,A′ to produce HMX or scission at B,B′ to produce Cyclonite (RDX).

When nitric acid containing nitrogen pentoxide was treated with DPT at 0° to 25° C. a linear compound, 1,9-dinitroxy-2,4,6,8-tetranitro-2,4,6,8-tetrazanonane, III, was obtained on dilution. Evidence for this structure is furnished by replacement of the nitroxy groups by methoxy or ethoxy radicals. This conversion of nitrate ester to alkyl ether is novel and specific for esterified amino methylols. It is remindful of the ester interchange recently reported (3), between dinitroxy and diacetoxy dimethylnitramide. The nitrate ester

[1] *Manuscript received November 16, 1948.*
Contribution from the Department of Chemistry, University of Toronto, Toronto, Ont.
[2] *Holder of a Studentship under the National Research Council of Canada, 1941–42.*

III also undergoes this ester interchange with sodium acetate in acetic acid to give 1,9-diacetoxy-2,4,6,8-tetranitro-2,4,6,8-tetrazanonane, II. This further substantiates the structure of III because it provides an acetyl analysis.



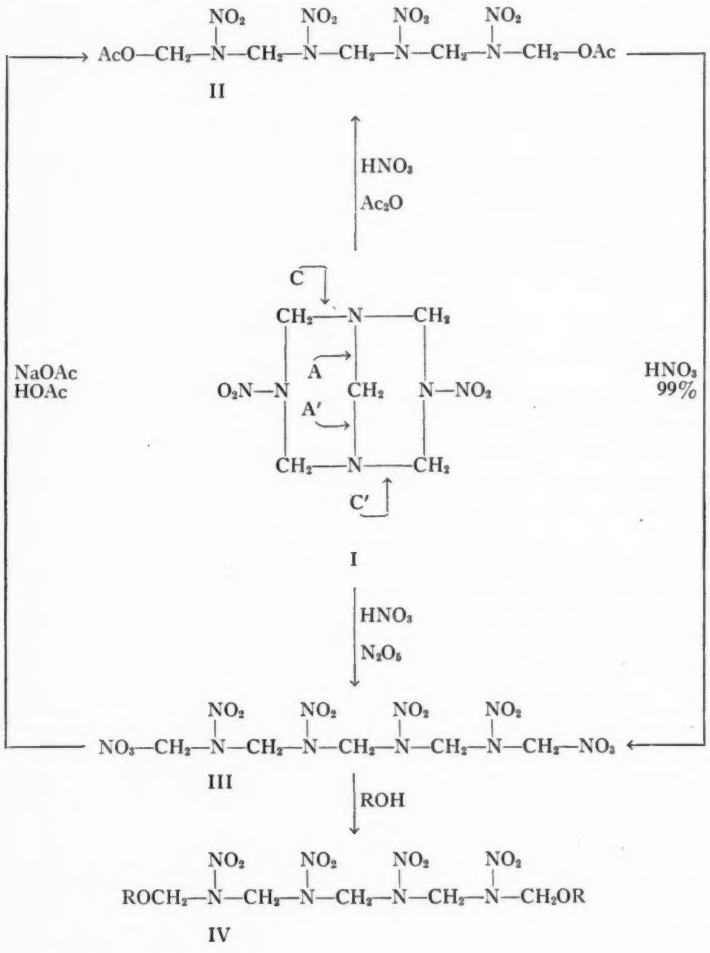
The acetate ester II can be produced directly and in good yield from DPT if the latter compound is treated at 44° C. with a mixture of acetic anhydride and nitric acid. It will ester-interchange in nitric acid solution to the dinitroxy compound III, and the two-step reaction is preferred for preparation of III because it obviates the requirement for nitrogen pentoxide.

The yield of 1,9-diacetoxy-2,4,6,8-tetranitro-2,4,6,8-tetrazacyclononane, II, is slightly better at 44° C. than at 65° C. but the course of the reaction at the higher temperature is the same. If ammonium nitrate is added to the nitric acid – acetic anhydride system, with which DPT is treated at 65° to 70° C. then the chief product is HMX. It is impure, and may be contaminated with 1,9-diacetoxy-2,4,6,8-tetranitro-2,4,6,8-tetrazanonane, II, but when it is purified from boiling nitric acid a 65% yield of good quality HMX is obtained.

It has been fashionable to consider a mixture of acetic anhydride and nitric acid as a source of acetyl nitrate, the ester being considered as the active agent. Evidence is accumulating, however, to indicate that acetyl nitrate is not active in such a mixture (4). Indeed the equilibrium $AcONO_2 + HOAc \longrightarrow Ac_2O + HNO_3$ seems to be far to the right. This has been tested in the procedure for conversion of hexamine to 1,5-dinitro-3,7-*endo*methylene-1,3,5,7-tetrazacycloöctane (DPT) (3) by replacing nitric acid with acetyl nitrate. In those experiments where acetic acid was present, DPT was obtained, although the yield was dependent on the amount of this acid used. However, when chloroform or acetic anhydride was used as the solution medium, only hexamine dinitrate could be discovered at the end of the reaction.

The significance of acetyl nitrate was also tested by repetition of the nitrolysis of hexamine to 1,9-diacetoxy-4-aceto-2,6,8-trinitro-2,4,6,8-tetrazanonane (2). When acetic acid was present a 16% yield was obtained,

almost identical with that obtained when acetic anhydride and nitric acid were used. Replacement of the acetic acid by acetic anhydride reduced the yield to 3%. This small yield was undoubtedly owing to the presence of



some acetic acid in the anhydride, since when either acetic acid or its anhydride was replaced by chloroform, none of the diacetoxyacetotrinitrotetrazanonane was obtained.

There seems then to be no evidence that acetyl nitrate acts as a nitrolyzing agent except when it has been converted by acetic acid to nitric acid and acetic anhydride. In the present discussion, this nitric acid – acetic anhydride mixture like nitrogen pentoxide in nitric acid, is considered a simple solution

wherein absence of water has appreciably reduced the acidity of the nitric acid. In both instances nitric acid is considered as the nitrolyzing agent, while acetic anhydride or nitrogen pentoxide are considered as esterifying agents.

In order to form the linear compounds II and III a different pair of scissions must occur than could be specified for the cyclic compounds HMX and Cyclonite (RDX). There are only two possibilities if structures are excluded in which two nitro groups are attached to one amino nitrogen, and no such compound has ever been reported. Either a symmetrical scission at C and C′ may occur, or else an unsymmetrical scission at A′ and C. No phase of the present work can suggest a choice, but Bachmann's conversion (1) of 1-acetoxymethyl-3,5,7-trinitro-1,3,5,7-tetrazacycloöctane (the intermediate he isolated in conversion of DPT to HMX) to the acetate ester II strongly suggests the unsymmetrical scission A′ and C.

On this basis, the weakest linkage during nitrolysis is probably a bridge-link; subsequently the choice may depend on the rapidity and completeness with which the free hydroxyl group is esterified. If, following scission at A′, the pendulant hydroxyl group remains free for an appreciable time, the subsequent split is at A, and liberation of formaldehyde accompanies the nitration. If the hydroxyl group is esterified, however, then the linkage A is strengthened, and subsequent scission will occur at the only available linkage, C.

If esterification alters the reaction to favor formation of the linear compounds, then the production of HMX instead of 1,9-diacetoxy-2,4,6,8-tetranitro-2,4,6,8-tetrazanonane when ammonium nitrate is present in the acetic anhydride – nitric acid solution must mean either that ammonium nitrate facilitates formaldehyde removal (demethylolation) or else hinders esterification.

The effects are probably intercorrelated. Thus, it is known that, when ammonium nitrate is dissolved in the medium, the reaction temperature must be raised to 60°–70° C. before the nitric acid is effective either for esterification or nitrolysis. This higher temperature undoubtedly promotes demethylolation. The freed formaldehyde can then be stabilized by methylolamine formation with the ammonium nitrate which is present in the medium.

Experimental*

1,9-Diacetoxy-2,4,6,8-tetranitro-2,4,6,8-tetrazanonane, II

To a stirred solution of 20 gm. (0.091 mole) DPT, I, in 438 cc. (4.66 moles) of acetic anhydride, was added 140 cc. (3.34 moles) of 99.6% nitric acid over 40 min. at 44° C. The mixture was then cooled to 15° C. and poured on ice. The filtered precipitate was washed with ethanol and air-dried to yield 31.7 gm., m.p. 176° to 185° C., or 87% of theoretical. Three crystallizations from

* *All melting points are corrected against reliable standards.*

nitromethane or, better, from acetone raised this to 186.5°–187.2° C. Calc. for $C_9H_{16}N_8O_{12}$: C, 25.2; H, 3.76; N, 26.2; CH_3CO, 19.8%. Found: C, 25.3; H, 3.93; N, 26.2; CH_3CO, 20.3%.

The acetyl determination was complete in one hour, thus indicating absence of the N—$COCH_3$ linkage. The high hydrogen result is characteristic of these unstable nitramines when the sample is burned without admixture with potassium dichromate. The compound is destroyed (82%) by one hour's reflux with boiling aqueous ammonia. The evaporated solution gives a positive lanthanum nitrate test (5) for acetic acid.

The compound gives a strong Franchimont test for the nitramine linkage. Its melting point is not depressed by the analogous nitrate ester, III (solid solution?). It is oxidized after 18 min. by 70% nitric at 25° C., but immediate dilution yields a compound melting poorly at 202° C. which has not been identified. It is neither Cyclonite nor III.

The substance can also be prepared from 1,9-dinitroxy-2,4,6,8-tetranitro-2,4,6,8-tetrazanonane, III. A solution of 0.3 gm. (7×10^{-4} mole) of III with 0.23 gm. sodium acetate in 5 cc. of acetic acid was boiled for three minutes, then cooled, filtered, and the precipitate washed with water. It weighed 0.2 gm. (70% of theoretical) and melted at 183° C. When Cyclonite was treated similarly it was recovered unchanged, thus indicating that the nitramino group is unaffected by such treatment.

1,9-Dinitroxy-2,4,6,8-tetranitro-2,4,6,8-tetrazanonane, III

When 150 gm. (0.36 mole) of pure diacetoxy compound II (m.p. 185° C.) was added to 1 liter (24 moles) of 99.6% nitric acid at 0° C., and the reaction mixture after 15 min. at 25° C. was poured into ice, a precipitate was formed. It weighed 153 gm. and melted at 202° to 203° C. This 98% yield was thrice crystallized from nitromethane to melt at 204.5° to 205° C., when the sample was introduced at 190° C. and heated rapidly. Less pure II gives an inferior product. Only 20% of the compound was destroyed in 70% nitric acid at 25° C. for 30 hr., but rapid decomposition occurred when the nitric acid was boiled. Calc. for $C_5H_{10}N_{10}O_{14}$: C, 13.8; H, 2.30; N, 32.3%. Found: C, 13.8; H, 2.43; N, 32.3%.

This compound is a powerful explosive (Trauzl block expansion 2.2 × TNT at bulk density 0.69). It is 12 times as sensitive to impact as TNT.

The same compound is obtained when DPT is treated with nitric acid containing enough nitrogen pentoxide to give an apparent titration of 106% HNO_3 (1.8 gm. 100% HNO_3 per gram N_2O_5). Ten grams (0.046 mole) of DPT was added to 63 cc. (1.6 moles) of stirred 106% nitric acid over 35 min. at 20° C. After a total time of 50 min. the whole was drowned in ice, filtered, water washed, and air-dried. The yield of 13.4 gm. (72% of theoretical) melted at 191° to 193° C. Three crystallizations from nitromethane raised this to 202° C.

1,9-Dimethoxy-2,4,6,8-tetranitro-2,4,6,8-tetrazanonane, IV. ($R = CH_3$)

When 0.2 gm. (4.6×10^{-4} mole) of the nitrate ester, III, was boiled in 10 cc. methanol for 10 hr., a compound crystallized out on cooling. When filtered and washed with methanol, it weighed 0.16 gm. and melted at 177° to 183° C. This 93% yield was crystallized from 1 : 1 dioxane–methanol to melt at 182° to 183° C. Calc. for $C_7H_{16}N_8O_{10}$: C, 22.6; H, 4.32; N, 30.8%. Found: C, 22.5; H, 4.27; N, 30.7%.

1,9-Diethoxy-2,4,6,8-tetranitro-2,4,6,8-tetrazanonane, IV ($R = C_2H_5$)

A procedure identical with the above gave a 43% yield of product, m.p. 166° to 167° C. The melting point was not raised by 1 : 1 ethanol–dioxane crystallization. Calc. for $C_9H_{20}N_8O_{10}$: C, 27.0; H, 5.04; N, 28.0%. Found: C, 27.4; H, 4.94; N, 28.2%.

1,3,5,7-Tetranitro-1,3,5,7-tetrazacycloöctane (HMX)

A mixture of 10 gm. (0.046 mole) of DPT (m.p. 203° to 204° C.) and 46.2 cc. (0.394 mole) of acetic anhydride was stirred at 65° to 70° C. (bath 60° C.) while a solution of 11.1 gm. (0.138 mole) of ammonium nitrate in 13.4 cc. (0.319 mole) of 99.6% nitric acid was added over a 15 min. period. The suspension dissolved and then reappeared. After subsequent stirring for 22 min. and 10 min. at 25° C., the whole was poured into 400 gm. of ice and water. The filtered solid, washed with 300 cc. water and air-dried, weighed 14.8 gm., m.p. 226° to 250° C. It was boiled with 207 cc. of 70% nitric acid until strong nitrogen oxide evolution commenced. After cooling and drowning with 2 liters water, it was filtered and dried at 70° C. to weigh 8.9 gm. (65.5% of theoretical) with melting point 267° to 268° C.

Cyclonite-HMX Mixture from DPT

A stirred solution of 1.1 gm. (0.014 mole) of ammonium nitrate in 1.3 cc. (0.031 mole) of 99.6% nitric acid was heated to 70°–75° C. in a water bath while 0.200 gm. (9×10^{-4} mole) of DPT was added. After 15 min. the mixture was drowned in ice and water, filtered, and the precipitate washed and dried. It weighed 0.15 gm. and melted at 187.5° to 189.5° C. According to thermal analysis by the melting point composition diagram to be reported in the next paper of this series, this contained about 30% HMX, which would constitute a 17% yield; the remainder, if Cyclonite, would be 52% of the theoretical both on the 1 : 1 basis. Fractional crystallization from nitromethane yielded these two, and no other, compounds.

This ratio of HMX to RDX was shifted by decrease in amount of nitric acid (34 moles to 20 moles) and increase of ammonium nitrate to quantity equimolar with the acid. A solution of 126 gm. of 99.6% nitric (2 moles) and 160 gm. (2 moles) of ammonium nitrate was stirred at 68° C. while 21.8 gm. (0.1 mole) of DPT was added over 20 min. After 30 min. more at this temperature, the mixture was drowned in ice and water and filtered. The product melted at 197° to 202° C. and weighed 13 gm. and, according to

thermal analysis, contained about 3% HMX. The yields are therefore 1.77% HMX and 57% Cyclonite. Neutralization of the liquors produced only a trace (less than 0.15%) of impure DPT.

When hexamine was subjected to exactly these same reaction conditions a good Cyclonite (m.p. 202° to 203° C.) was obtained in 19% yield after purification from hot 70% nitric acid. Neutralization of the reaction liquors precipitated a 17% yield of DPT on the equimolar hexamine basis.

Attempted Formation of DPT and 1,9-Diacetoxy-4-aceto-2,6,8-trinitro-2,4,6,8-tetrazacycloöctane, XVI, with Acetyl Nitrate

The acetyl nitrate was prepared by the method of Pictet and Khotinsky (6); it boiled at 38° to 43° C. (15 mm.). The procedures wherein this reagent replaced nitric acid – acetic anhydride were otherwise identical with those reported elsewhere (2, 3) except when chloroform was used in the preparation of DPT (Expt. 4, Table I). In this instance a gummy solid precipitated from the chloroform solution. This seemed to be a complex of the type which acetyl nitrate forms with pyridine. It was intractable until it was dissolved in aqueous acetic acid, from which hexamine dinitrate was precipitated. The proportions and yields are indicated in Table I.

TABLE I

Expt. No	Moles hexamine	Moles HOAc	$CHCl_3$, cc.	Moles HNO_3 99%	Moles Ac_2O	Moles $AcNO_3$	Product
1	0.014	0.688			0.024	0.028	DPT, m.p. 204° C. Yield, 36%
2	0.014	0.145			0.024	0.028	DPT, m.p. 199° C. Yield, 12%
3	0.015		15			0.029	Hexamine dinitrate, Yield, 66%
4	0.015				0.106	0.029	Hexamine dinitrate, Yield, 21%
5	0.014	0.13			0.01	0.048	XVI, m.p. 148° to 151° C. Yield, 16%
6	0.014	0.13		0.048	0.058		XVI, m.p. 146° to 148° C. Yield, 15%
7	0.014				0.01	0.048	XVI, m.p. 149° to 150° C. Yield, 3%
8	0.014		15			0.048	No water-insoluble product

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NITROLYSIS OF HEXAMETHYLENETETRAMINE

III. PREPARATION OF PURE CYCLONITE[1]

By F. J. Brockman, D. C. Downing,[2] and George F Wright

Abstract

All Cyclonite prepared by nitrolysis of hexamethylenetetramine has been found to contain 1,3,5,7-tetranitro-1,3,5,7-tetrazacycloöctane (HMX). This impurity is not present in Cyclonite prepared by oxidation of 1,3,5-trinitroso-1,3,5-triazacyclohexane. The oxidation yields 1-nitroso-3,5-dinitro-1,3,5-triazacyclohexane as an intermediate. There seems then to be a difference in reactivity of two of the three aza linkages in triazacyclohexanes.

When Cyclonite is prepared from hexamethylenetetramine (hexamine) with nitric acid containing acetic anhydride (1, 4), it always contains 1,3,5,7,-tetranitro-1,3,5,7,-tetrazacycloöctane, HMX, as an impurity. The extent of this impurity can be estimated by thermal analysis according to the melting point – composition diagram shown in Fig. 1. The lower line of this diagram represents the first observable softening of the sample, while the top line records the disappearance of the last crystal.

Cyclonite prepared by the method of Hale (2) ordinarily melts at 202° to 203.5° C. after it has been boiled with nitric acid. According to Fig. 1 it ought then to contain less than 1% of impurity. Although the impurity cannot be detected by microscopic crystallographic examination it must necessarily be present, because repeated wasteful crystallization of Cyclonite from acetic acid finally raises the melting point to a constant value of 204.5° to 205° C.

Confirmation that this impurity was HMX was obtained during an inspection of one of the wartime plants which manufactured Cyclonite by the Hale method. A scaly deposit on the apron of a classifier filter had formed where the diluted reaction mixture was cooled, and this turned out to be almost pure HMX (m. p. 269° C.). It must, therefore, be concluded that all Cyclonite prepared from hexamine contains HMX as an impurity; this impurity is difficult to remove because it is more stable and less soluble than Cyclonite.

In view of the uncertainty concerning purity of a substance which tends by its rapid crystallization to occlude impurities strongly, a method was sought whereby Cyclonite could be prepared otherwise than by nitrolysis of hexamine. It was found that this could be accomplished by oxidation of 1,3,5-trinitroso-1,3,5-triazacyclohexane, I.

The trinitroso compound, I, is prepared from hexamine (3) in aqueous solution. Although it is not very stable it can be purified with ease from the impurity 1,5-*endo*methylene-3,7-dinitroso-1,3,5,7-tetrazacycloöctane, which is formed at the same time. The trinitrosotriazacyclohexane, I, reacts

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Contribution from the Department of Chemistry, University of Toronto, Toronto, Ont.
[2] *Holder of a Studentship under the National Research Council of Canada, 1941-42.*

violently with strong nitric acid at room temperature, usually with inflammation, but it does not char at −40° C. if it is added to the strong acid in small portions.

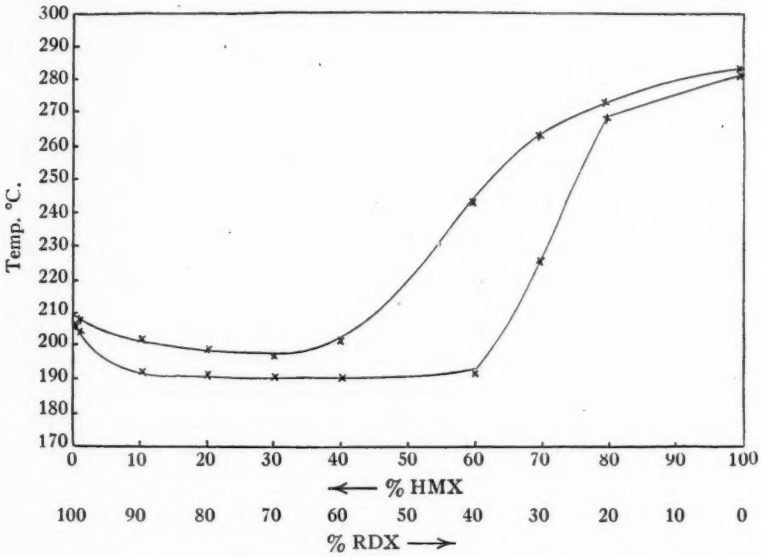


FIG. 1. *Curve showing complete range of RDX–HMX mixed melting points.*

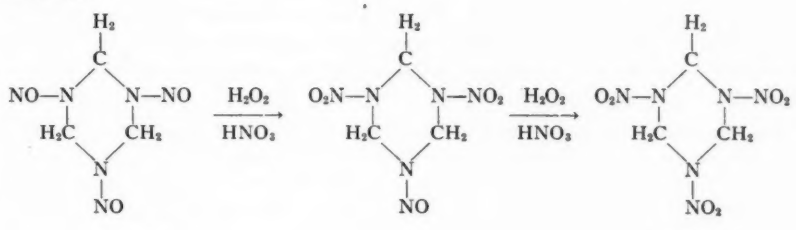
The oxidizing medium first used consisted of 82 equivalents of 99% nitric acid, 3 of hydrogen peroxide, and 3.7 of water (introduced with the peroxide, thus reducing the nitric acid concentration to 92–94%). This solution was chilled to −40° C. and one molar proportion of the trinitrosamine added carefully in small portions, with vigorous stirring. The reaction mixture probably absorbed water from the air during this addition.

When the clear reaction mixture at −40° C. was poured on to ice, a light yellow solid precipitated which was not Cyclonite. It gave positive Franchimont and Liebermann tests for nitro and nitroso groups, respectively, and it liberated iodine from acid potassium iodide, although more slowly than did the trinitrosamine, I. Cyclonite liberates almost no iodine from aqueous potassium iodide. When the product was returned to the same oxidizing mixture at −40° C. and the solution allowed to warm to room temperature, it was converted to very pure Cyclonite in good yield.

These properties, together with the analytical data, indicate that the compound II is 1-nitroso-3,5-dinitro-1,3,5-triazacyclohexane.

The compound II is contaminated with Cyclonite (RDX) but is sufficiently soluble in boiling water that it can be separated from this impurity, and no indication has been found that a mononitro-dinitroso analogue is present.

This indicates that when two nitro groups have been established in the cyclotrimethylenetriamine ring the third is introduced with somewhat more difficulty.



I II RDX

The isolation of dinitronitrosotriazacyclohexane, II, depends on relative reaction rate and not on deficiency of oxidizing agent. This may be shown by allowing the reaction mixture to stand overnight at −40° C. Dilution on ice yields 54% of the theoretical amount of Cyclonite (RDX) instead of the 49% yield of II which can be obtained if the reaction solution is drowned shortly after addition is complete. If, alternatively, when addition at −40° C. is complete, the solution is at once allowed to warm to room temperature, the product is pure Cyclonite and not II.

Reaction conditions indicating the optima are shown in Table I. It may be seen that the reaction may be carried out without hydrogen peroxide

TABLE I

SYNTHESIS OF CYCLONITE

Expt. No.	Equiv. HNO_3	Moles, I	Addn. time, min.	Equiv. 30% H_2O_2	Additional variation	Holding time, min. at			Yield crude RDX	
						−40° C.	0° C.	30° C.	%	M.p.
1	80	0.001	5	3	0	0	500	0	55	204.5
2	240	0.001	5	3	0	0	500	0	46	204
3	85	0.187	30	3	0	0	1000	0	65	205.0
4	85	0.187	30	3	0	0	1000	1000	67	203
5	85	0.011	15	0	3 eq. Na_2O_2	15	0	25	47	205
6	85	0.011	15	1.5	0	10	0	1000	64	205
7	85	0.011	15	0.75	0	10	0	1	66	205
8	85	0.011	15	0.75	0	10	0	60	66	205.0
9	85	0.011	15	0.75	0	10	0	1000	63	205.0
10	85	0.011	15	0.75	Run at 0° C.	0	0	120	10	205.0
11	85	0.011	15	0.75	Run at 0° C.	0	0	1000	12	205
12	85	0.011	15	0	0	0	0	10	66	202.5
13	85	0.011	15	0	0	0	0	1000	63	203.5
14	43	0.011	30	0	0	0	0	5	74	204
15	22	0.011	20	0	0	0	0	5	53	200
16	22	0.011	40	0	0	10	0	5	52	198.5
17	43	0.011	35	0	0.05 eq. $AgNO_3$	0	0	5	69	204.5
18	43	0.01	25	0	0.05 eq. V_2O_5	0	0	5	71	203
19	43	0.011	30	0	No water	0	0	5	48	202.5
20	43	0.011	35	0	No water	0	0	5	57	202.5

(Expts. 12 to 20) but the product is less pure. This may be owing to the beneficial effect of the water introduced in the peroxide. Van Romburgh (5) recommends 89% nitric acid for oxidation of dinitro-*p*-tolylmethylnitrosamine to the nitramine. Strictly anhydrous reaction conditions (Expts. 19 and 20) seem to give poorer product than was obtained from Expt. 14 under react on conditions which were identical except for the exposure to atmospheric moisture.

The unusual purity of these crude products can be appreciated only after attempting to purify rigorously any Cyclonite prepared by nitrolysis of hexamine. The nature of the oxidation process may be expected to exclude possibility of eight-membered ring formation. The high purity is undoubtedly owing to absence of HMX.

Experimental*

Purification of Cyclonite

The best explosive-grade Cyclonite melts at 201.5° to 202.5° C. Cyclonite melting at 204.5° to 205.0° C., can be obtained by five crystallizations from acetic acid. In order to realize the high melting point, the product which crystallizes in massive crystals, extremely sensitive to impact, must be ground (under water) to crack open the faults and pockets which frequently occur. It is presumed that this acetic acid crystallization removes only 1,3,5,7-tetranitro-1,3,5,7-tetrazacycloöctane (HMX) because this is the only other substance of the methyleneamine type which will resist oxidation by nitric acid. It is wise, prior to the acetic acid crystallization, to crystallize impure Cyclonite from boiling 70% nitric acid in an open flask.

1,3,5-Trinitroso-1,3,5-triazacyclohexane, I

To a stirred solution of 105 gm. (0.75 mole) hexamine in 1400 cc. water plus 600 gm. ice, in a 5 liter three-necked flask, was added an ice-cold solution from 340 cc. (4.1 moles) of concentrated hydrochloric acid and 1 kilo of ice. The acid solution was stirred while a solution of 250 gm. (3.6 moles) of sodium nitrite in 250 cc. water plus 150 gm. ice was added. The reaction mixture foamed up immediately; the foaming was controlled by air streams directed at the necks of the vessel. After one-half hour without stirring, the precipitate which had risen to the surface was filtered off, washed with water, and dried at 45° C. to give 61.1 gm. (26% on CH_2O basis) of pale yellow powder, m. p. 105° to 107° C. Of this, 60 gm. was crystallized from boiling ethanol (9 cc. per gm.) to give 48.8 gm., m. p. 105° to 107° C.; a second crop of 6.8 gm. was recovered on evaporating to 70 cc.

1-Nitroso-3,5-dinitro-1,3,5-triazacyclohexane, II

A solution was prepared by adding 3.9 cc. (*ca.* 0.035 mole) of 30% C. I. L. hydrogen peroxide dropwise to 41.5 cc. (62 gm., 0.99 mole) of 99% nitric

* *All melting points are corrected against reliable standards. The heating rate for Cyclonite samples was 2° C. per minute.*

acid stirred at −40° C. Stirring was continued at this temperature while 2.0 gm. (0.012 mole) of the powdered trinitrosamine was added in small portions over 15 min. No flashes of flame were observed, and only a little white fume. The clear yellow liquid was stirred for 30 min. longer at −40° C. and poured (still clear yellow with no sign of char) on 120 gm. of ice. The reaction mixture smelled of nitrous acid but not of formaldehyde. The light yellow solid which precipitated was filtered, and air-dried to weigh 1.12 gm. (45% of theoretical on basis of I → II) and to melt at 125° to 137° C., depending on rate of heating. Neutralization of the filtrate with ammonia to pH 5.6 yielded no trace of precipitate.

This compound is very soluble in acetone, nitromethane, and dioxane; quite soluble in chloroform and slightly less so in ethanol and benzene. Its solubility in water is less than in the above, but is quite appreciable. Crystallization from boiling benzene (50 cc. per gm.), and subsequently from boiling water (110 cc. per gm.), leaves much of the contaminant RDX undissolved. This material, m. p. 160° to 165° C., is then finally purified by solution in a minimum of hot nitromethane. The RDX crop which separates on cooling is filtered off and the solution diluted with an equal volume of ether. The crystal crop thus obtained melts at 173° to 174° C.; repetition of this process yields a product melting at 176.6° C. (decomp.). Calc. for $C_3H_6N_6O_5$: C, 17.5; H, 2.91; N, 40.8%. Found: C, 17.6; H, 2.97; N, 40.5%.

In boiling mineral acids (for example, 15% nitric acid) it is decomposed with evolution of formaldehyde, easily detected by odor; on boiling with 10% sodium hydroxide a faint odor of ammonia is observable and a bromthymol blue test paper held in the mouth of the test tube is turned blue immediately. The compound gives a positive Franchimont test. In the Liebermann test it dissolves in cold concentrated sulphuric acid without immediate char or visible gassing and on addition of a phenol crystal does not change color; on warming, a green color develops. Cyclonite treated similarly develops a yellow color in the cold which goes to green on warming. The compound liberates iodine from aqueous potassium iodide faster than Cyclonite (which indeed does not appear to liberate iodine appreciably at room temperature) but much slower than trinitrosotrimethylenetriamine. In a semi-quantitative test, 0.02 gm. each of trinitrosotrimethylenetriamine, the oxidation product being investigated, and RDX, along with a blank control were each suspended in 2 cc. water plus 2 drops glacial acetic acid, and 0.10 gm. of solid potassium iodide added. The trinitrosamine tube was bright yellow in two minutes, the oxidation product pale yellow in 27 min., at which time the Cyclonite and blank tubes were each a barely perceptible yellow of intensity identical to the eye.

1,3,5-Trinitro-1,3,5-triazacyclohexane (RDX)

Dinitronitrosotriazacyclohexane, II, can be converted to RDX by further treatment with hydrogen peroxide – nitric acid. When 0.05 gm. of II was added at −40° C. to 0.10 cc. of 30% hydrogen peroxide in 1 cc. of 99% nitric

acid and the reaction allowed to warm to room temperature and maintained there for five minutes, 0.04 gm. (75% of theoretical) of very pure RDX, m. p 205° C., was obtained by pouring the reaction mixture on 4 gm. of ice. A mixed melting point with purified RDX, m. p 204.5° C., was not depressed.

The isolation of the intermediate is possible only when the cold reaction mixture (−40° C.) is poured on ice without being allowed to warm to room temperature. Thus the 45% yield described in preparation of II involved a 30 min. period at −40° C.; when the solution at this temperature was poured on ice immediately after addition was complete, the yield was 49%. When this reaction mixture was allowed to stand at 0° C. overnight, the product isolated after pouring on ice was a 54% yield of RDX, m. p. 205° C. When threefold quantities of hydrogen peroxide and nitric acid were used under these latter conditions, the yield was 45%.

The one-stage process standardized for the experiments listed in Table I consisted in addition of powdered I added (except in Expts. 19 and 20) to the strongly stirred nitric acid solution in a wide-mouth Erlenmeyer flask in a bath at −35° to −40° C. The Cyclonite was precipitated when the solution was poured into ice, filtered, ground, and dried at 100° C.

Oxidizing agents which failed to effect the conversion are: nitric acid – ammonium nitrate, nitric-phosphoric acids, nitric-acetic acids, nitric acid – acetic anhydride, camphor nitrate, tetranitromethane, Caro's acid, aqueous potassium chlorate, neutral hypochlorite, manganic acetate, ferric chloride, and nitric acid in nitromethane.

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